Potomac River at Knoxville, Maryland. Photo credit Jim Cummins

Biological Surveys of Three Potomac River Mainstem Reaches (2012-2014) with Considerations for Large River Sampling

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Disclaimer

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Executive Summary

The Interstate Commission on the Potomac River Basin (ICPRB) conducted a study to describe the biological composition of three under-represented reaches in the mainstem Potomac River Basin and determine the effort required to accurately assess large river sites for freshwater mussel and benthic macroinvertebrate populations. Located at Knoxville (MD), Carderock (MD), and Little Falls (MD), these reaches were selected because they are difficult to sample and represent gaps in spatial coverage of the mainstem in the otherwise comprehensive Maryland Department of Natural Resources (MD-DNR) Core Trend Monitoring Program. Data from the Knoxville reach will improve our understanding of the mixing zones below the confluence of the Shenandoah and Potomac rivers and the relative importance of each river at the Potomac water supply intakes downstream. The Carderock and Little Falls reaches are important in identifying stresses on the river's biological communities that could relate to upstream consumptive losses and water supply withdrawals during severe droughts. The Little Falls reach is in the only stretch of the Potomac River with a minimum flow-by requirement.

Surveys of freshwater mussel and benthic macroinvertebrate populations were conducted during late-summer low-flow periods of 2012, 2013, and 2014. The three years of the study had moderate flows overall and did not experience extreme drought or floods, so managers and researchers should view the results as a characterization of biological communities unaffected by flow extremes. In addition to recording mainstem Potomac species distributions, biological collections underwent post-collection analyses that provided an informed baseline for the collection effort required to achieve sufficiently accurate data in the future.

Freshwater mussels Collections yielded 875 individuals across the three mainstem reaches during the 2012-2014 period. Four species were identified: Eastern Elliptio (*Elliptio complanata*), Lamp mussels (*Lampsilis sp.**), Brook Floater (*Alasmidonta varicosa*), and the Creeper (*Strophitus undulates*). Average detection rates ranged from a low of 3.06 mussels/person-hour at Knoxville to a high of 44.70 mussels/person-hour at Carderock, with detections at Little Falls falling in between at 24.50 mussels/person-hour. Mussel densities were 0.05 mussel/m^2 at Knoxville, 0.44 mussels/m^2 at Little Falls, and 0.49 mussels/m^2 at Carderock. As freshwater mussels are one of the most imperiled groups in the United States, the significant effort required to document their presence in the mainstem Potomac River was worth the logistical hardships these methods entail.

Benthic macroinvertebrate Community composition was similar across all three reaches. Carderock and Little Falls locations had similar taxa richness values (72 and 66 genera, respectively) while the upstream Knoxville location had a somewhat higher taxa richness (87 genera). A genus level inventory of mainstem benthic macroinvertebrates was recorded for each location. A stepwise rarefaction analysis was applied post collection to the raw datasets to calculate catch per unit effort (CPUE) for each of the large river reaches. Our results show that 400-count subsampling is applicable to richness level metrics in large river locations, while metrics such as percent composition, diversity metrics, and tolerance metrics require less effort (100-count subsampling). A non-metric multidimensional scaling (NMDS) analysis was applied to benthic samples to confirm that during the three sampling years (2012-2014) there was no significant differences observed due to inter-annual variation.

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Introduction

Study Goals and Objectives

The Potomac River mainstem is a boundary for parts of Maryland, Virginia, West Virginia and the District of Columbia. The river's tributaries are monitored by resource agencies of the four jurisdictions; its mainstem is monitored primarily by the Maryland Core/Trend program. Sections of the lower free-flowing mainstem, between Harper's Ferry and Washington, DC, are difficult to access and are thus poorly sampled.

ICPRB has observed and evaluated the Potomac River and its tributaries since the Commission formed in 1940. Recognizing the need for more data and information about the mainstem of the Potomac River, ICPRB initiated this study aimed at characterizing biological communities in three under-represented mainstem reaches. The purpose is to enhance the basin jurisdictions' collective ability to assess the mainstem's biotic condition with robust and scientifically-defensible datasets. The desired outcome is improved water quality assessments and water resource management of the mainstem Potomac River.

The goals of this study are:

(1) Augment existing data and information about the lower Potomac River mainstem with surveys at three under-represented reaches: Knoxville, Carderock, and Little Falls.

(2) Determine the sampling effort required to characterize these biological communities at the three sites with a high degree of certainty.

(3) Contribute to a baseline dataset that will inform future studies about the impacts on the freshwater mussels and benthic macroinvertebrates of extreme flows.

Approach and Rationale

Flowing waters become harder to sample as they move from creeks and small rivers into large rivers, and their biological communities and habitats gradually change in what has been described as the River Continuum Concept (Vannote et al. 1980). Large rivers are deeper, wider, and less affected by terrestrial canopy cover than their smaller tributaries. Underlying abiotic variables such as geology, climate, hydrology, and topography further affect large river biological communities, and can result in significant differences between neighboring large rivers. Biological communities in large rivers across the United States are consequently not as well described as their wadeable tributary and stream counterparts (Flotemersch, 2006). Although there have been advancements in the technologies and methodologies used to study large rivers, biological sampling methods continue to be a target for researchers and policy makers alike (Royer et al 2001, Wessell et al. 2008, Blocksom and Johnson 2009, Weigel and Dimick 2011).

Freshwater mussels and benthic macroinvertebrates were the biological communities selected for study because they serve important roles and functions in large river ecosystems. Individuals are typically not able to move quickly and usually cannot avoid the stresses impacting a location. Freshwater mussels and benthic macroinvertebrates were surveyed at three locations in the lower Potomac River mainstem, the largest section of the river, to improve the understanding of ecological conditions in that section. The three study locations are difficult to access and two have not been routinely sampled. They represent gaps in the current understanding of the river. Timed visual search and excavations of the substrate inside a sampling frame were used to sample mussels; the commonly used D-shaped kick net method was used to sample benthic macroinvertebrates.

Information about the three study reaches may be able to aid in drinking water spill response and water resources management. Data from the Knoxville reach will improve our understanding of the mixing zones below the confluence of the Shenandoah and Potomac rivers and the relative importance of each river at the Potomac water supply intakes downstream. The Carderock and Little Falls reaches are especially important in identifying stresses on the river's biological communities that could be related to upstream consumptive losses and water supply withdrawals during severe droughts. The Little Falls reach is in the only stretch of river with an environmental flow-by requirement. A minimum flow of 100 million gallons per day (mgd) was established by the Potomac River Environmental Flow-by Study (MD DNR, 1981) and implemented through the 1978 Potomac River Low-Flow Allocation Agreement.

Study Area

The Potomac River is the second largest tributary to the Chesapeake Bay. In total, the Potomac River drains about 14,700 square miles (38,073 km^2) from four states (Maryland, Pennsylvania, Virginia, and West Virginia) and the District of Columbia. The river's mainstem, from the confluence of the North and South Branch Potomac rivers to Great Falls, flows nearly 170 miles (271 km) southeast, cutting through the Appalachian Mountains and Piedmont provinces and becoming tidal as it enters the Coastal Plain near Washington D.C. The Potomac River mainstem can be divided into four sections: the upper free-flowing, middle free-flowing, lower free-flowing, and estuarine/tidal (Figure 1). The study's three survey locations are in the lower free-flowing Potomac segment.

Definitions vary as to what constitutes a large river (Flotermersch 2006). The lower Potomac river segment selected by ICPRB for this study is generally considered a large river. It has a large watershed (roughly 24,000 km^2), is classified as Strahler stream order 7, is non-wadeable in many reaches, and most of its surface is not shaded by the riparian canopy.

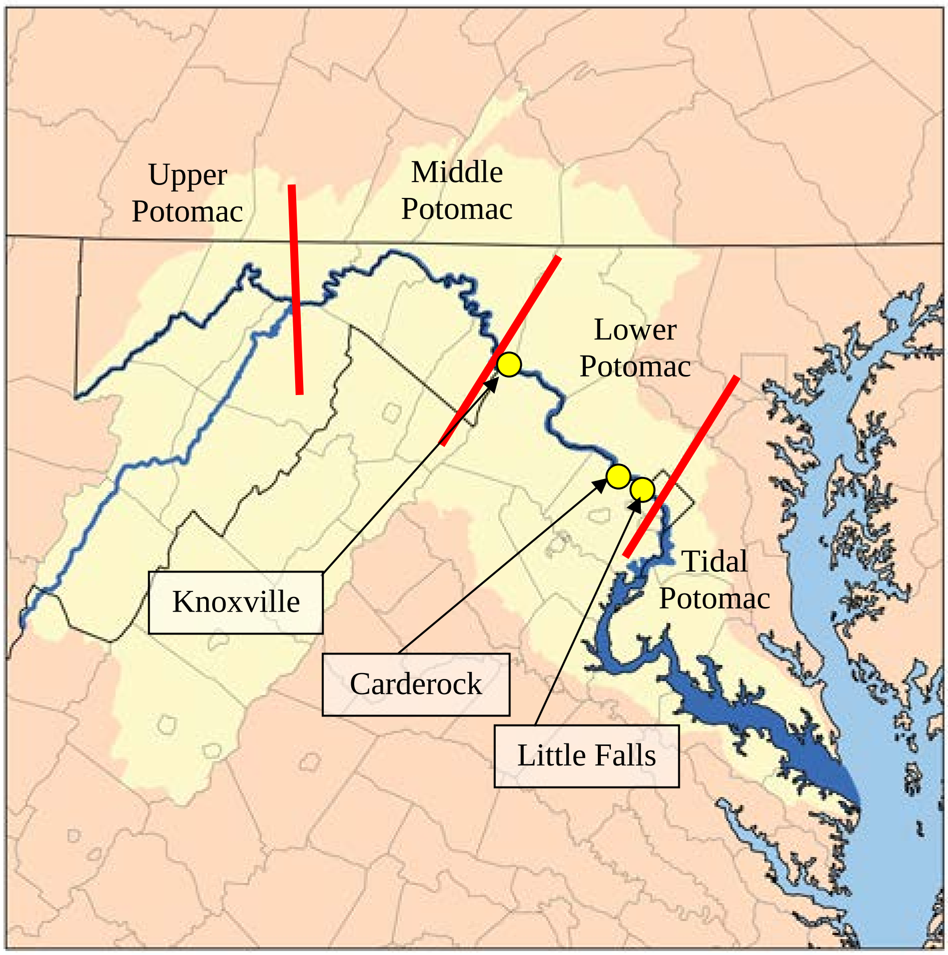

Figure 1. Study locations on the Potomac River mainstem. The Potomac Basin (light yellow) with the Potomac River segments indicated: Upper Potomac, Middle Potomac, Lower Potomac, and the Tidal Potomac.

Survey locations are intended to help fill gaps in the spatial coverage of the otherwise comprehensive Maryland's Core Trend monitoring program (Figure 2). Once appropriate locations were identified on a macroscale, possible study reaches were narrowed to those having all four key macrohabitats: riffle, run, pool, and glide. Due to logistical and budgetary constraints, only three reaches were ultimately selected in the lower Potomac river at Knoxville, Carderock, and Little Falls. Stream confluences, bridges, or known pollutant sources did not occur near any of the reaches. Access to each required canoe transport of field personnel and equipment. Poor accessibility is the primary reason these reaches are underrepresented in the historical data. The central coordinates and descriptions of the watersheds upstream of the three reaches are given in Table 1.

Few dams regulate flow in the streams and large rivers of the Potomac River basin compared to other eastern U.S. river systems. Most of the 481 impoundments identified in the watershed are run-of-river facilities that minimally alter flow patterns (USACOE 2013). Of the three study reaches, Little Falls is the only study reach that is immediately impacted by a dam, which is located 100 m upstream. The Little Falls dam is used to withdraw raw drinking water from the river for the metropolitan Washington DC area.

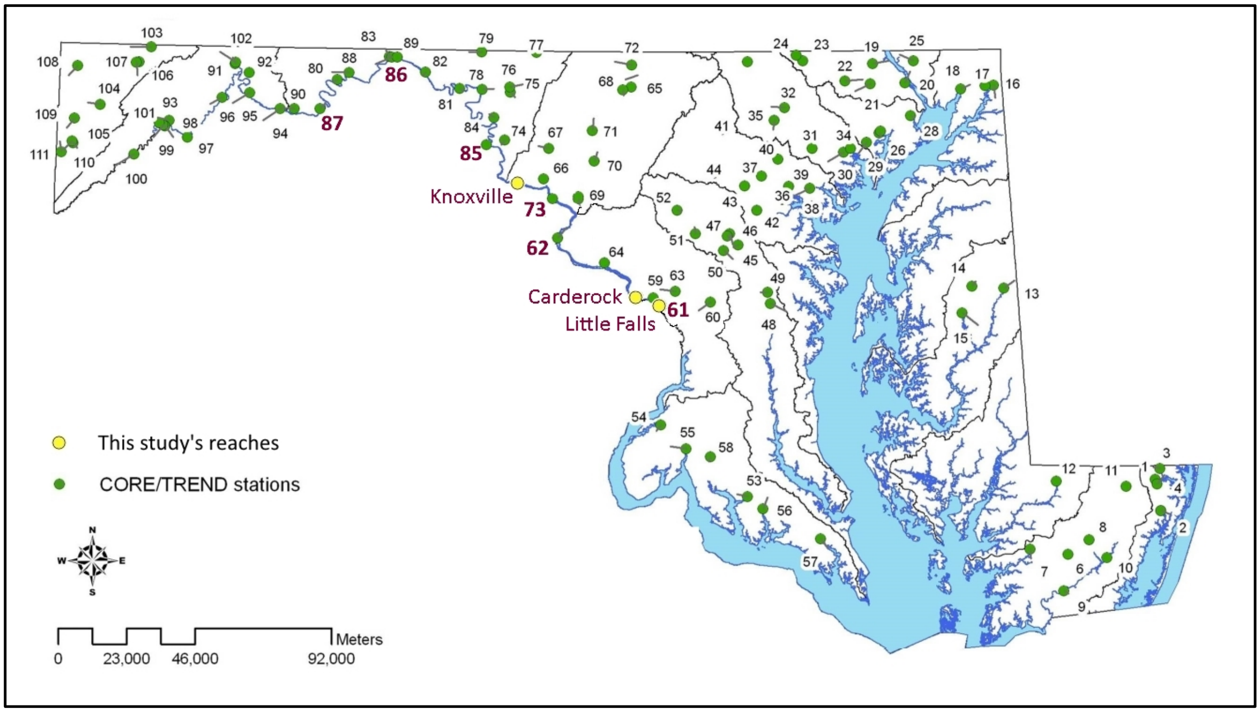

Figure 2. Map of Maryland Core/Trend benthic monitoring sites adapted from Friedman (2009). Yellow dots indicating the three ICPRB large river study reaches. Of the 111 Maryland Core/Trend stations, only six sites are on the mainstem Potomac River. The ICPRB Little Falls study reach is a Maryland Core/Trend station (#61). The other Core/Trend stations on the Potomac River mainstem are: Whites Ferry (#62), Point of Rocks (#73), Shepherdstown (#85), Hancock (#86), and Paw Paw (#87).

Table 1. Location coordinates and descriptions of watersheds upstream of the three study reaches, including drainage area, reach area, and land uses (derived using Model My Watershed Tool https://app.wikiwatershed.org/analyze.)

	Knoxville	**Carderock**	**Little Falls**
Coordinates	39.327266° N, 77.671952° W	38.968772° N, 77.196064° W	38.948710° N, 77.129294° W
Upstream Drainage Area	24,356 (km^2)	29,874 (km^2)	29,978 (km^2)
Study Reach Length	1.27 (km)	0.93 (km)	0.81 (km)
Study Reach Area	126 acres (0.51 km^2)	57 acres (0.23 km^2)	52 acres (0.21 km^2)
Average Gradient	2.09 m:km	0.76 m:km	3.99 m:km
Deciduous Forest	13,958.07 km^2 (57.6%)	15,512.65 km^2 (52.2%)	15,539.59 km^2 (52.1%)
Pasture/Hay	5,318.64 km^2 (21.9%)	6,778.94 km^2 (22.8%)	6,779.54 km^2 (22.7%)
Cultivated Crops	942.73 km^2 (3.9%)	1,955.35 km^2 (6.6%)	1,955.13 km^2 (6.6%)
Developed, Open Space	1,277.17 km^2 (5.3%)	1,883.75 km^2 (6.3%)	1,923.8 km^2 (6.4%)
Evergreen Forest	1,004.11 km^2 (4.1%)	1,103.78 km^2 (3.7%)	1,105.54 km^2 (3.7%)
Developed, Low Intensity	533.24 km^2 (2.2%)	816.6 km^2 (2.7%)	835.82 km^2 (2.8%)
Mixed Forest	649.63 km^2 (2.7%)	709.34 km^2 (2.4%)	711.6 km^2 (2.4%)
Developed, Medium Intensity	151.17 km^2 (0.6%)	282.72 km^2 (1.0%)	289.25 km^2 (1.0%)
Open Water	169.72 km^2 (0.7%)	216.42 km^2 (0.7%)	218.63 km^2 (0.7%)
Woody Wetlands	28.92 km^2 (0.1%)	134.11 km^2 (0.5%)	135.32 km^2 (0.5%)
Shrub/Scrub	56.89 km^2 (0.2%)	90.44 km^2 (0.3%)	86.99 km^2 (0.3%)
Barren Land (Rock/Sand/Clay)	25.75 km^2 (0.1%)	86.56 km^2 (0.3%)	84.73 km^2 (0.3%)
Developed, High Intensity	73.17 km^2 (0.3%)	84.86 km^2 (0.3%)	92.96 km^2 (0.3%)
Grassland/Herbaceous	43.11 km^2 (0.2%)	62.42 km^2 (0.2%)	62.57 km^2 (0.2%)
Emergent Herbaceous Wetlands	13.14 km^2 (0.1%)	20.84 km^2 (0.1%)	20.91 km^2 (0.1%)
Nearest Upstream Impoundment	26.7 mi/ 42.9 km (Potomac) 8.5 mi/ 13.7 km (Shenandoah)	4.5 mi/ 7.2km (Potomac)	0 mi/ 0 km (Potomac)

Methods

Study reach boundaries were established from satellite images and later confirmed in pre-sampling visits. A grid composed of 5m x 5m (25 m^2) numbered cells was superimposed on the satellite images and random number generator was used to identify grid cells for mussel sample collections. The center point of each selected cell was identified in the field using hand-held GPS (Garmin model Etrex 20). Data on macrophytes—filamentous algae and submerged aquatic vegetation (SAV)—were collected in the grid cells. Additional random cells were visited to further characterize macrophytes in each reach. Figure 3 and Appendix 1 show examples of the grid system at the Little Falls reach. Each study reach was also divided into four approximately equal-sized quadrants, and benthic macroinvertebrates were collected in the best riffle-pool-riffle sequence in each quadrant.

ICPRB collected biological data between the months of August and October in 2012, 2013, and 2014. Best effort was made to completely sample each study reach within a two-week window, weather allowing. Only one exception to the continuity of sampling occurred in 2012 where the Potomac Basin experienced significant rainfall, which elevated river flows to unsafe levels and pushed back completion of Carderock sampling to late October (Table 2). This delay caused incomplete mussel counts as diving was no longer possible in late October.

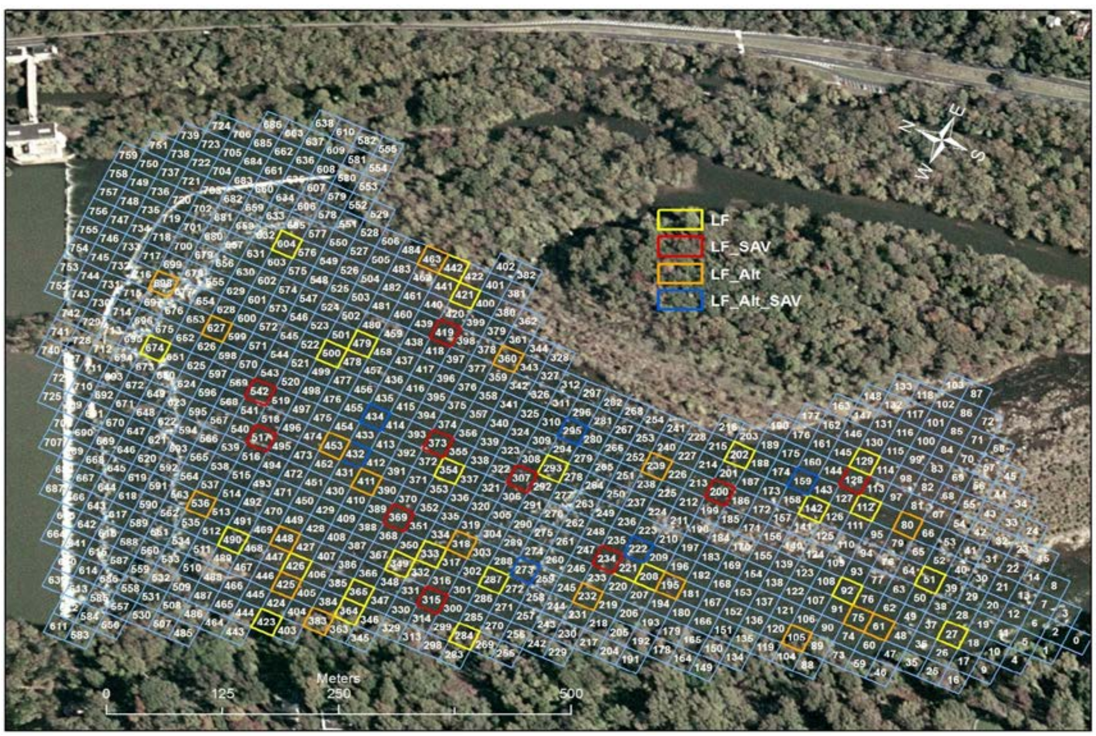

Figure 3. Example of grid of 5m x 5m cells overlain on the Little Falls reach. Yellow boxes are grid cells randomly assigned for the mussel survey; red boxes are grid cells randomly assigned for the submerged aquatic vegetation (SAV) random survey; orange and blue boxes are randomly identified alternates if the assigned grid cells were deemed inappropriate (e.g. dewatered areas, scoured bedrock, etc.).

Table 2. Dates of sampling events for each study reach. *The desired 2-week sampling window was exceeded at Carderock in 2012 due to excessive flows in the river and no SAV was reported.

Site	2012	2013	2014
Knoxville	8/23, 8/24, 8/31	8/6, 8/7, 8/8	9/12
Carderock	9/17, 10/26*	8/19, 8/22	8/11
Little Falls	9/6, 9/7, 9/14	9/18	8/29

Freshwater Mussels

Mussels were sampled by 1) excavating the substrate inside a sampling frame located at the center point of each randomly selected grid cell, and 2) performing a timed visual search in a 12.50 m² area around the sampling frame. Additional random grid cells were identified as alternates to use if the assigned grid cell was deemed unsamplable (e.g., dewatered, high risk to staff).

Mussels were collected from each of 25 randomly selected grid cells (5 m x 5 m). Habitat parameters were recorded, including depth, flow characterizations, estimates of substrate composition, stream morphology, embeddedness, and coverage estimates of submerged aquatic vegetation (SAV) and filamentous algae. ICPRB staff used a blindly tossed ¼ m² sampling frame (Figure 4) to subsample each grid cell as excavation of the entire 25 m² grid cell would have been logistically infeasible. The sample frame area was first visually examined for mussels and then excavated to a depth of approximately 15 cm. Sand, gravel, gobble and any mussels from the excavations were placed into a 0.25 m² collection box with a 1 cm² (0.375 in²) wire-mesh bottom, then removed to a canoe for further examination.

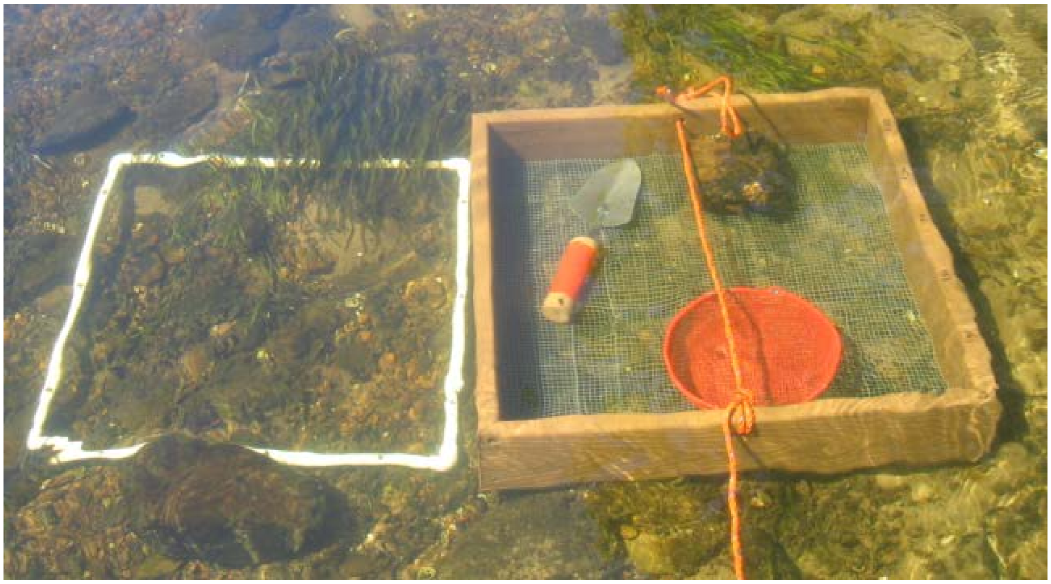

Figure 4. The ¼ m² sampling frame (white) and collection box (brown) used in mussel surveys.

Excavation samples are quantitative measures for estimating the relative occurrence of buried species or individuals that would otherwise be overlooked with solely visual searches (Strayer 1997, Obermeyer 1998, Strayer 2003). The excavation sample in each grid cells was followed by a timed qualitative visual search performed in a 2m radius circle (12.50 m^2 area) centered on the sampling frame. The timed qualitative visual searches were used to better estimate mussel species richness and relative abundance, and to aid in detecting rare species. Mussels encountered during the subsequent circle search were kept separate from the excavation samples. All mussels were kept in shaded containers with fresh river water until they had been identified, measured (length, width, and height), recorded (see Appendix 2), after which they were placed back in the river in their approximate original location and orientation. Digital images were made of selected mussels for vouchers or to document anomalies. Results of the excavation collections and timed visual searches were used to develop taxa richness, density, and abundance measures.

Benthic Macroinvertebrates

There is little research on the required reach length for adequately sampling macroinvertebrates and other fauna in large rivers (but see Flotemersch et al. 2006). Due to this absence of guidance, the benthic macroinvertebrate sampling design for this study was fashioned after a riffle-pool-riffle sequence (Lyons 1992), modified from large river vertebrate sampling methods. Correlations reported between invertebrate communities and fish assemblages in other large rivers (Kilgore and Barton 1999) justifies to some extent the use of the large river vertebrate methods for sampling macroinvertebrates. The three Potomac River study reaches all had a riffle-pool-riffle complexes, which allowed a standardized sampling strategy. We were also able to confirm the presence of diverse macrohabitat assemblages to sample within the complexes.

Macroinvertebrate samples were collected in riffle-pool-riffle sequences using a 500-micron mesh D-shaped kick net (50 cm wide x 30 cm tall x 60 cm deep with removable seine plastic purse). This method is commonly used in shallower streams and using it in the larger mainstem makes the results easier to comparable to upstream sites. The method differs from the Fullner modified Hester-Dendy multi-plate sampler that was routinely used by the Maryland Core/Trend Program at all of its Potomac River mainstem stations. Consistent use of the multi-plate sampler can facilitate trend analyses, but the data are not directly comparable to data collected with the various kick net samplers (Friedman 2009, personal comm).[1]

Each of the three study reaches was divided into four quadrants and the best available riffle-pool-riffle habitats in each quadrant was identified and sampled. Quadrants were identified as upper left (UL), upper right (UR), downstream left (DL), or downstream right (DR). In each quadrant, a composite of six 0.25 m^2 areas was collected for a total collection area of 1.5 m^2. Kicks were performed in the standard manner, i.e., moving substrate by kicking with the feet, except in 2014, when, due to injury, a triangular shaped Warren hoe was used to move the substrate. This modification in side by side comparisons in the field yielded similar effects to substrate disturbance, however, should not be considered as a defensible alternative in the future.

[1] Maryland Core/Trend Program uses the Fullner modified Hester-Dendy multiplate sampling method at its Potomac mainstem monitoring stations due to depths greater than 0.46m and lack of shallow riffles. Samples also were collected simultaneously with the Hester-Dendy multi-plate sampler for several years.

Samples were preserved in the field in 70+% alcohol (95% EtOH diluted by captured invertebrates and organic matter in the collection bag), with labels both in and on the container. Samples were transferred to ICPRB storage and laboratory facilities for subsequent sorting and laboratory identification, enumeration, and data entry. Each quadrant (UR, UL, LL, LR) collection was evenly distributed in a standard sorting pan with 28 equally-sized sub-sampling grid cells. The 28 grid cells were given identification numbers 1-28 and randomly selected for sorting. From the randomly selected grid cells, two 100 ± 20% organism count samples (“Sample 100A,” “Sample 100B”) and one 200 ± 20% organism count sample (“Sample 200C”) were created, for a total count of 400 individuals picked per river quadrant (400 ± 20%, “Sample 400D”). The total count for all four quadrants was about 1,600 organisms. Laboratory identifications were performed to highest level of identification, most often genus level, by either ICPRB staff or contracted taxonomists with EcoAnalyst (website or contact information for EcoAnalyst inserted here). Chironomides and oligochaetes were rounded up to family and class, respectively, to control for inconsistencies in ID between years. Taxa preparation R-scripts for species, genus, and family round up definitions can be observed in Appendix 3.

To estimate subsampling efficacy, the four river quadrants samples (400D) were combined into a single 1,600 count whole reach sample (1600E). It was assumed that organisms were distributed randomly throughout the four quadrants and thus the 1,600-count sample could be considered random as well. Subsample simulations were run in R (Rstudio 3.3.0 “Another Canoe”). A random sample without replacement function (100 iterations) was used to simulate different subsampling efforts at each 100-organism fixed count size from 100 to 1,600 subsample group. Due to two subsampling rules (± 20% of target value stipulation and complete counting of the selected grid), there are some cases where there were as many as 1,900 organisms or as few as 1,200 organisms representing the four-quadrant composite sample. In both cases, the maximum fixed organism count was used to run the iterative model.

Macrophytes

Submerged aquatic vegetation (SAV) and filamentous algae coverage were recorded in each sampled macroinvertebrate grid cell (Figure 5). Ten randomly selected master grid cells (defined separately from benthic sample grid cells) in each reach were also evaluated. In each master grid cell, the central point of the cell anchored a 25 m linear transect radiating outward 12.5m toward each bank from each assigned grid cell’s central point. The linear transects were used to record species and measurements of the length of line covering individual species clusters (in 0.1 m increments) to derive diversity and percent coverage (see

Figure 5. Jim Cummins conducts a 25m linear transect assessing macrophyte distribution at the Knoxville site. In the foreground is a cluster of stargrass (Heteranthera dubia) in bloom.

Appendix 4). During this study, only macroalgae and submerged vascular plants were considered as periphyton identification was too costly to consider.

Flow Conditions

Flow analyses were calculated using NWIS daily mean flow data from October 1, 2011 through Sept 30, 2014 USGS for Little Falls (USGS 01646500) and Point of Rocks (USGS 01638500) USGS gage stations. Analyses was conducted using U.S. Geological Survey "water years," a temporal definition of flow defined by the 12-month period spanning October 1 of a given year through September 30 of the following year. The use of a water year in flow analyses more accurately captures the flow regime experienced by annually recruited macroinvertebrate taxa and aligns with the sampling dates of this study. The use of calendar years was considered but was determined to not completely capture macroinvertebrate cohort structure correctly (removal of fall flows on autumn laid eggs and late season instars).

Five commonly used flow metrics were calculated from daily mean flow data: the 1-day maximum, 3-day maximum, pulse count, flashiness, and rise rate (Table 3). A flow index developed by Olson (2005) was also applied to obtain a relative measure of each season and water year's flows. The Olson Flow Index classifies flows by river into one of seven categories based on a 1975 – 1994 baseline period of flows measured at the USGS Point of Rocks gage (01638500). Mean daily flows (cubic feet per second) are multiplied by 60 seconds/minute * 60 minutes/hour * 24 hours/day to obtain cubic feet per day, and then summed over all days of a given season or year. The Olson Flow Index classifies these seasonal and yearly cumulative flows into one of seven categories based on what was experienced during the 20-year baseline period: Record Dry (less than the baseline minimum), Very Dry (less than 10th percentile), Dry (10^{th} – 33^{rd} percentile), Moderate (33^{rd} – 67^{th} percentile), Wet (67^{th} – 90^{th} percentile), Very Wet (greater than 90^{th} percentile), Record Wet (greater than baseline maximum).

Table 3. Definitions of flow metrics.

Metric name	Description
1 Day Maximum	The average of each water year's highest daily mean flow divided by catchment area for a twenty-year period.
3 Day Maximum	The average of each water year's highest 3-day mean flow divided by catchment area for a twenty-year period.
Pulse Count	The median of the annual average of each water year's number of times the daily mean flow is above the 90^{th} percentile of all the flows for a twenty-year period.
Flashiness	The absolute values of all day-to-day changes in daily mean flows are summed for the entire study period and divided by the sum of all the daily mean flows (Richards-Baker Index)
Rise Rate	The average of all positive differences in daily mean flow during 'rising periods', or consecutive days for which the change in daily flow is positive, in a water year divided by catchment area.
Olson Flow Index	Daily mean flows [in cubic feet per second] are multiplied by the constant (60 second/minute *60 minutes/hour * 24 hours/day) to obtain cubic feet per day, then summed over all days in a season or year. Cumulative flows are then classified according to Olson (2005) described above.

Results

Physical Habitat and Macrophytes

Depth, substrate composition, and SAV and filamentous algae coverage were recorded in each sampled grid cell. The depths of all the randomly located grids (n = 108) ranged from 4 inches (0.1 m) to 6.20 feet (1.9 m) with an average depth of 2.20 feet (0.6 m). Knoxville and Little Falls had nearly identical average sampled depths (0.64 m and 0.65 m, respectively), while Carderock was deeper at 0.82 m. All three reaches are fall areas, and therefore scour is a significant factor. The substrate at each reach was dominated by bedrock, boulders, cobble, and gravel (Table 4). Both Carderock and Little Falls are in the Potomac River Gorge, where the river is bound by bedrock banks and ledges which leads to the bottom substrate being substantially influenced by change in flow. Knoxville is located below the confluence of the Shenandoah and Potomac mainstem rivers where a broadening and general slowing of the river occurs. As a result, the Knoxville reach has slightly larger sections of deposited sand and silt between bedrock ledges. Other materials, like detritus and shells of bivalves, primarily *Corbicula* shells, made up small amounts of the substrate at all locations, most often in eddies and high flow refugia.

Table 4. Average depth and instream habitat composition, by reach.

Reach	Average Depth (m)	% Bedrock	% Boulders	% Cobble	% Gravel	% Sand	% Silt	% Other
Knoxville	0.6	15.1	9.0	30.5	22.15	13.4	6.6	3.5
Carderock	0.8	27.6	17.1	22.3	16.2	12.2	4.7	0.1
Little Falls	0.6	19.9	20.2	26.2	17.3	12.3	3.4	0.4
Overall Average	0.7	20.9	15.4	26.3	18.6	12.6	4.9	1.3

The percent cover of submerged aquatic vegetation and relative amount of periphyton were visually estimated in the ¼ m^2 sampling frame used to collect macroinvertebrates and at randomly located grid cells. In most instances, SAV coverage was less than 10% and periphyton was absent or present in low abundance (Table 5). SAV species documented in the survey were water stargrass (*Heteranthra dubia*) and water celery (*Vallisneria americana*). The Knoxville reach was the only reach with a large amount of SAV: 34.7% coverage in 2012 and 19.0% coverage in 2013. No macrophyte assessment was made in 2014. The Carderock reach had no SAV and the Little Falls reach had only 1.0% Stargrass and 3.4% submerged American water willow (*Justicia americana*), the latter is technically not an SAV species, but an emergent grass.

Filamentous green algae (FGA) was not routinely encountered at any of the three reaches during this study; however, patches of muskgrass (Chara spp.) were found at levels sufficient to limit habitat quality at two grid cells in the Knoxville reach on the side most influenced by Shenandoah River inputs. There were no obvious differences in habitat types or water chemistry that would help explain why algal blooms manifest on this side and not the other. In addition, the

Knoxville reach contained several areas outside of randomly selected grid cells which were heavily impacted by blue-green algae.

Table 5. Average coverage of submerged aquatic vegetation (SAV), filamentous green algae, and periphyton at the three study reaches. SAV cover was visually estimated as a percent of the sampling frame area. Periphyton in the sampling frame area was visually classified into one of four subjective categories: 0 (none), 1 (low), 2 (medium), and 3 (high).

Reach	Year	% Star Grass	% Water Celery	% Musk-grass	% Submerged Water Willow	% Grids with No SAV	Periphyton (0 – 3)
Knoxville	2012	31.2	3.5	0.08	0.02	65.1	0.7
Knoxville	2013	11.0	8.0	0.0	0.0	81.0	0.5
Carderock	2013	0.0	0.0	0.0	0.0	100.0	0.0
Carderock	2014	0.0	0.0	0.0	0.0	100.0	1.2
Little Falls	2012	0.9	0.0	0.0	2.7	96.4	0.0
Little Falls	2014	1.0	0.0	0.0	4.1	94.9	1.0

Flow Conditions

With few exceptions, the monthly median flows for each of the three water years in the study fell within the middle quartile of the recent twenty-year flow data (Figure 6). A high flow year (1996) and a drought year (1966) are shown in Figure 6 for comparison purposes. Five common flow metrics and a flow classification method were used to further characterize flow patterns during the water years represented in the study (Table 6).

The water year 2012 had the lowest flow metric values for both magnitude and rate of change. There was a slight increase in four of the five calculated flow metrics from WY2012 to WY2014. Water years 2013 and 2014 were more like each other than WY2012. Despite these differences, the similarities across the three years and between the two gage sites suggest there were no substantial temporal or spatial differences in hydrologic regime in the three study reaches.

Olson flow characterizations confirm that all three water years had moderate annual flows, indicating overall flows for those years were within the middle third of annual flows of the baseline period (1975 – 1994) for the Potomac's Point of Rocks USGS gage. WY2012 had a wet autumn and dry spring and WY2013 and WY2014 had wet springs, but all other seasons were moderate.

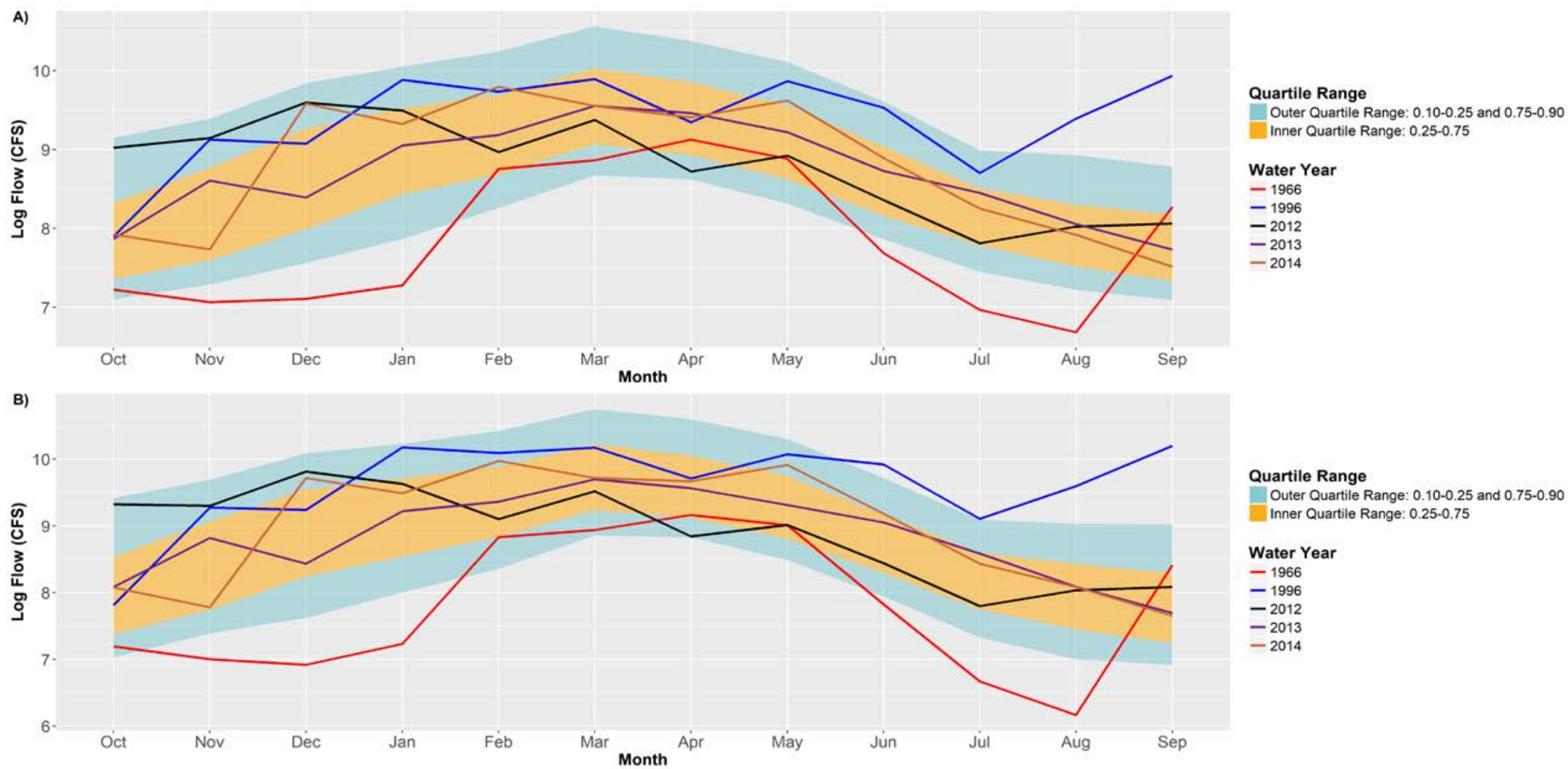

Figure 6. Median flow (log of adjusted CFS) for the Potomac River at (A.) Point of Rocks and (B.) Little Falls, Maryland, for the water years 2012-2014. Reference flows for drought year (1966) and flood year (1996) were included for comparison. Inner and Outer quartiles were calculated using all available historical data for each location; Point of Rocks, 1895-2014 and Little Falls, Maryland 1930-2014,

Table 6. Flow metric and Olson Flow Index calculations for water years 2012, 2013, and 2014. Values are derived from USGS daily mean flows at Point of Rocks, MD (01638500) and Little Falls, MD (01646500). Little Falls data are the observed flows and are not adjusted for the upstream withdrawals.

Location	Metric	WY 2012	WY 2013	WY 2014
Point of Rocks (USGS 01638500)	1-Day Max	4.50	9.95	13.68
	3-Day Max	5.20	6.45	9.22
	Pulse Count	43	46	67
	Flashiness	0.17	0.21	0.21
	Rise Rate	29.32	38.48	42.32
	Olson Characterization			
	Autumn (previous year)	Wet	Moderate	Moderate
	Winter	Moderate	Wet	Wet
	Spring	Dry	Moderate	Moderate
	Summer	Moderate	Moderate	Moderate
	Annual (water year)	Moderate	Moderate	Moderate
Little Falls (USGS 01646500)	1 Day Max	6.99	11.59	12.46
	3 Day Max	5.57	8.28	9.81
	Pulse Count (20Yr Average)	32	41	66
	Flashiness	0.18	0.24	0.22
	Rise Rate	29.74	43.36	46.95

Mussel Distribution and Abundance

Fourteen or fifteen native mussel species, depending on taxonomic classification, are recognized in the Potomac River basin (Cummins et. al. 2010). As this is one of the most imperiled groups of aquatic organisms in North America, we have included a mussel component in this study to record species distribution data and to associate observed riverine morphology with and flow-ecology relationships.

A total of 875 living mussels comprised of four species were collected at 108 mussel sites (Table 7). A total of 271 individuals were collected in 2012, 247 in 2013, and 357 in 2014. Mussels were not marked, therefore between years the same individuals could have been collected more than once in subsequent years. Carderock counts are only partial for 2012 due to high flows interrupting the survey. The Eastern Elliptio (*Elliptio complanata*) were especially abundant in the Carderock and Little Falls reaches. Lamp mussels (*Lampsilis* sp.) were found at each reach: they were slightly more abundant at the Knoxville reach. There are outstanding taxonomic issues with *Lampsilis* species, and the ones collected in the Potomac may be *L. cariosa*, *L. cardium*, hybrids between the two, or a native subspecies *L. cardium cohongoroton*. It is notable that evidence of a Maryland endangered species, the Brook Floater (*Alasmidonta varicosa*), was found at the Knoxville and Little Falls reaches. A sole living Creeper (*Strophitus undulates*), a Maryland rare species, was collected at the Knoxville site in 2012 and a fresh dead shell was found in the Knoxville study area in 2013.

Table 7. Observed freshwater mussel species collected in survey. FD, deceased individual.

Species	Knoxville (2012 – 2013)	Carderock (2012 – 2013 – 2014)	Little Falls (2012 – 2014)
Alasmidonta varicose (Brook Floater)	2 – 5	0	1(FD) – 1(FD)
Elliptio complanate (Eastern Elliptio)	9 – 9	48 – 226 – 164	192 – 192
Strophitus undulates (Creeper)	1 – 1(FD)	0	0
Lampsilis sp. (Lampmussel)	13 – 5	2 – 2 – 0	4 – 1, 1(FD)
Detection by time (# mussels/person-hour)	3.06 – 5.12	NA – 44.71 – 37.70	19.40 – 29.10
Density (# mussels/m²)	0.06 – 0.05	NA – 0.49 – 0.36	0.44 – 0.43

Benthic Macroinvertebrates

Despite the logistical difficulties of macroinvertebrate collection in a large river system dominated by bedrock, boulders, and cobble, both the kick method and hoe method statistically appear to be acceptable options for the lower free-flowing Potomac River. A total of 112 taxa were captured in the mainstem Potomac River between 2012 and 2014. Knoxville had the greatest taxa richness (87) followed by Carderock (72) and then Little Falls (66). In every reach, *Stenelmis* was the dominant taxon, followed by *Cheumatopsyche*, *Corbicula*, *Baetidae*, and/or *Isonychia* (Table 8). Ranked lists of all macroinvertebrate taxa from each reach, by year, are presented in Appendix 5.

Development of the catch curves from both raw capture data (Figure 7) and a random rarefied model of the accumulated data (Figure 8) showed similar trends in catch per unit effort (CPUE) across all three reaches. All CPUE catch curves show a rapid increase in taxa richness from 100 to 500 count samples. Beyond 500 organisms, the CPUE catch curve begins to level out and only infrequently found taxa are added with significant additional effort. Post analysis of the CPUE catch curves (Figure 9) looked at the percent change of each sorting group (100 organisms) by metric type. Richness metrics require more effort to achieve adequate precision than do percent metrics. Richness metrics require an effort of at least 400 sorted organisms to achieve less than a 5% change in metric score and 1,000 organisms to achieve a less than 2% change in taxa richness score. Percent metrics (metrics that rely on groups such as feeding groups, tolerance values, etc.) achieved less than 2% change in taxa percent accuracy at the 100-organism count.

Table 8. The top 40 most common taxa in the lower section of the non-tidal Potomac River. Cumulative for Knoxville, Carderock, and Little Falls and ranked by percent composition of the total population.

Rank	Taxon	Taxa Composition (Percent)	Rank	Taxon	Taxa Composition (Percent)
1	Stenelmis	34.34%	21	Helicopsyche	0.98%
2	Cheumatopsyche	8.48%	22	Protoptila	0.92%
3	Corbicula	7.54%	23	Orthotrichia	0.79%
4	Isonychia	4.26%	24	Oligochaeta	0.73%
5	Baetis	4.23%	25	Petrophila	0.73%
6	Gammarus	3.33%	26	Cnephia	0.60%
7	Hydropsyche	3.28%	27	Optioservus	0.56%
8	Tricorythodes	3.28%	28	Plauditus	0.56%
9	Anthopotamus	2.75%	29	Psephenus	0.54%
10	Macrostemum	2.24%	30	Teloganopsis	0.49%
11	Chimarra	2.10%	31	Neoperla	0.41%
12	Heterocloeon	2.07%	32	Neureclipsis	0.37%
13	Chironomidae	1.65%	33	Simulium	0.24%
14	Agnetina	1.56%	34	Microcylloepus	0.23%
15	Maccaffertium	1.53%	35	Bithynia	0.21%
16	Brachycentrus	1.47%	36	Acroneuria	0.20%
17	Argia	1.37%	37	Baetidae	0.18%
18	Leptoxis	1.34%	38	Acentrella	0.16%
19	Corydalus	1.13%	39	Platyhelminthes	0.14%
20	Leucrocuta	1.06%	40	Heptageniidae	0.13%

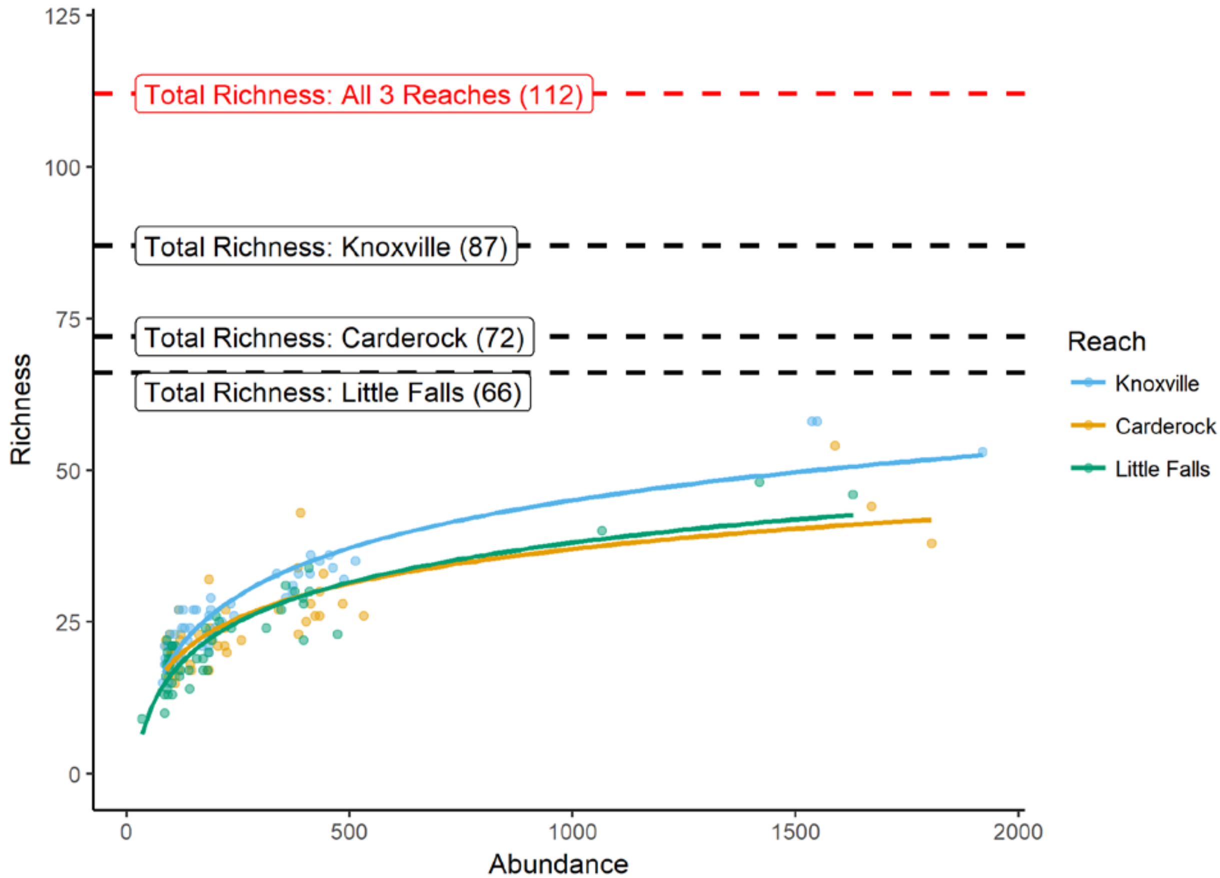

Figure 7. Catch curves developed from raw capture data for Knoxville, Carderock, and Little Falls, by year. "Total richness" is the compilation of all year and quadrant results. Catch curve represents logarithmic fit of raw collection data.

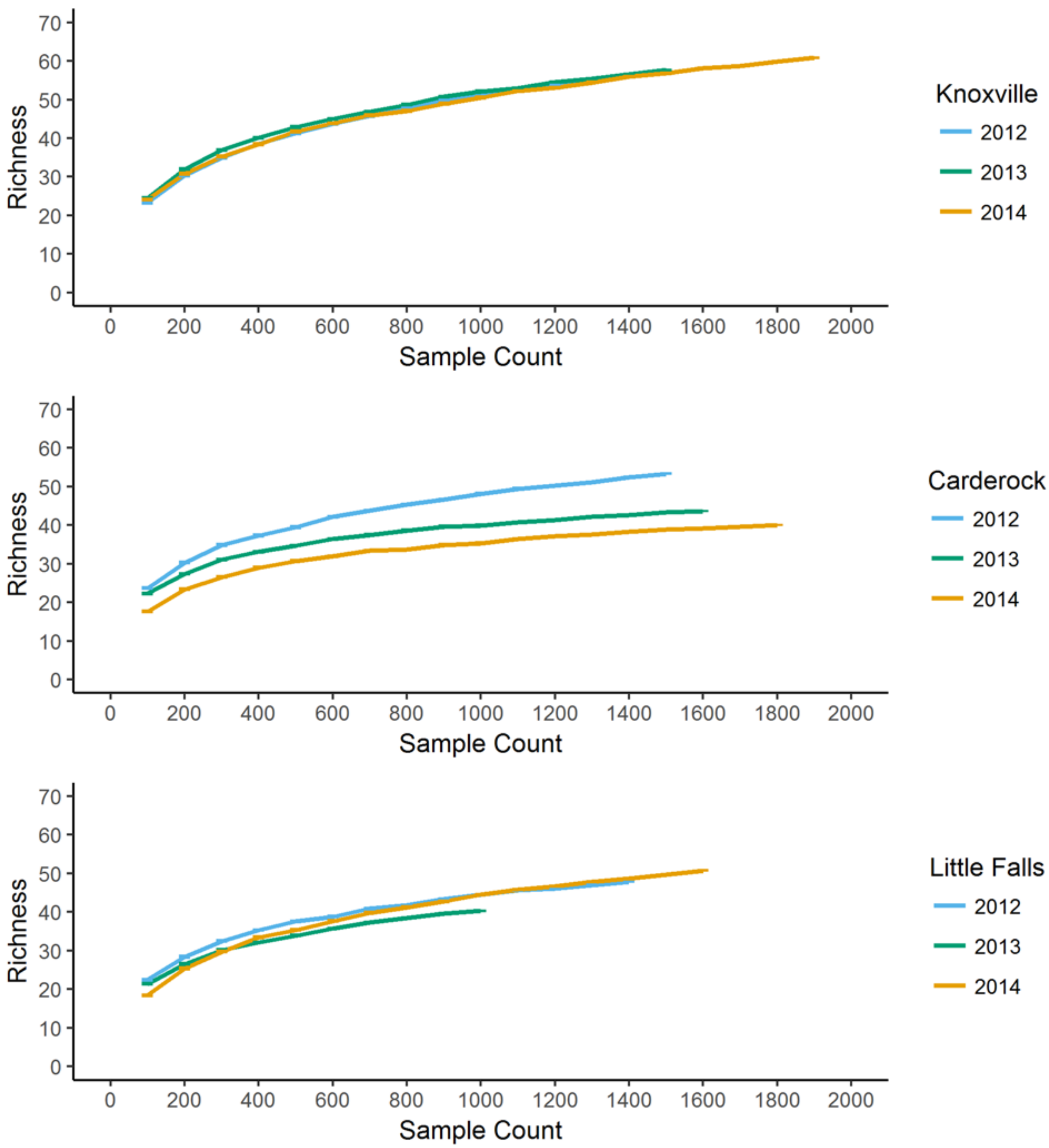

Figure 8. Predicted taxa richness catch curves by year for Knoxville, Carderock, and Little Falls using random rarefaction modeling of raw data for each 100 count sampling effort.

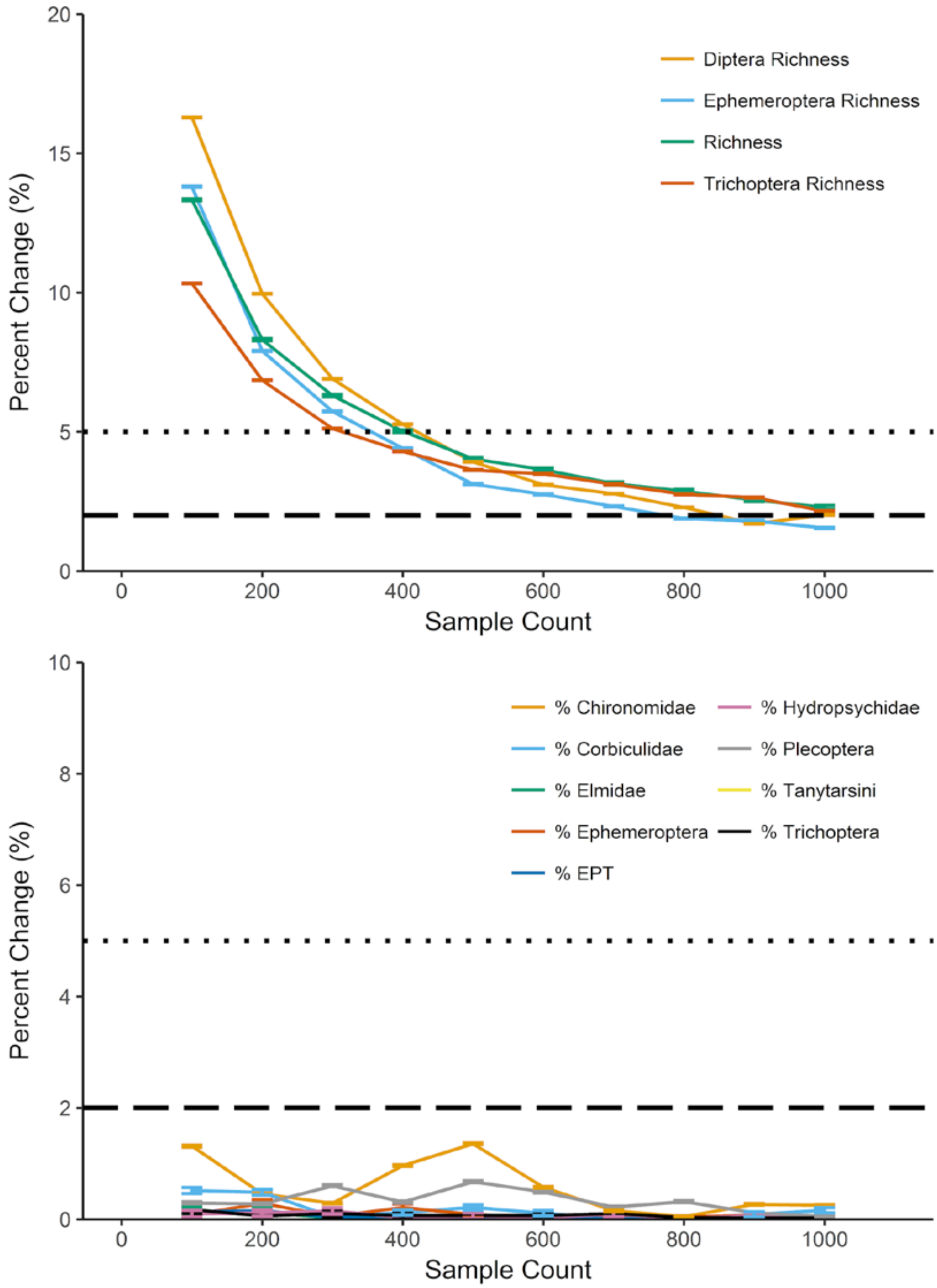

Figure 9. Percent change in metric scores between each subsample size. Richness metrics require a much greater effort (> 400 count) to reduce variation below 5% while percent metrics show very low variability (< 2%) at the 100-count level.

Nonmetric Multi-Dimensional Scaling (NMDS) was performed using the R package vegan's function "metaMDS" (Oksanen et al. 2018). The number of dimensions was set to four (k = 4) and the number of random starts was set to 1,000 (trymax = 1,000), otherwise the default settings for "metaMDS" were applied (i.e., distance measure used was Bray Curtis and the data were transformed using a square root transformation and Wisconsin double standardization). Twenty (20) random starts were necessary to find a convergent solution and the stress was 0.16. McCune et al. (2002) suggest that NMDS stress less than 0.20 generally provides an accurate representation of the data. The 95% confidence interval ellipses around each year's NMDS analysis centroid suggested that the communities collected each year are comparable across both time and location (Figure 10) and the assumption of homogeneous communities is supported.

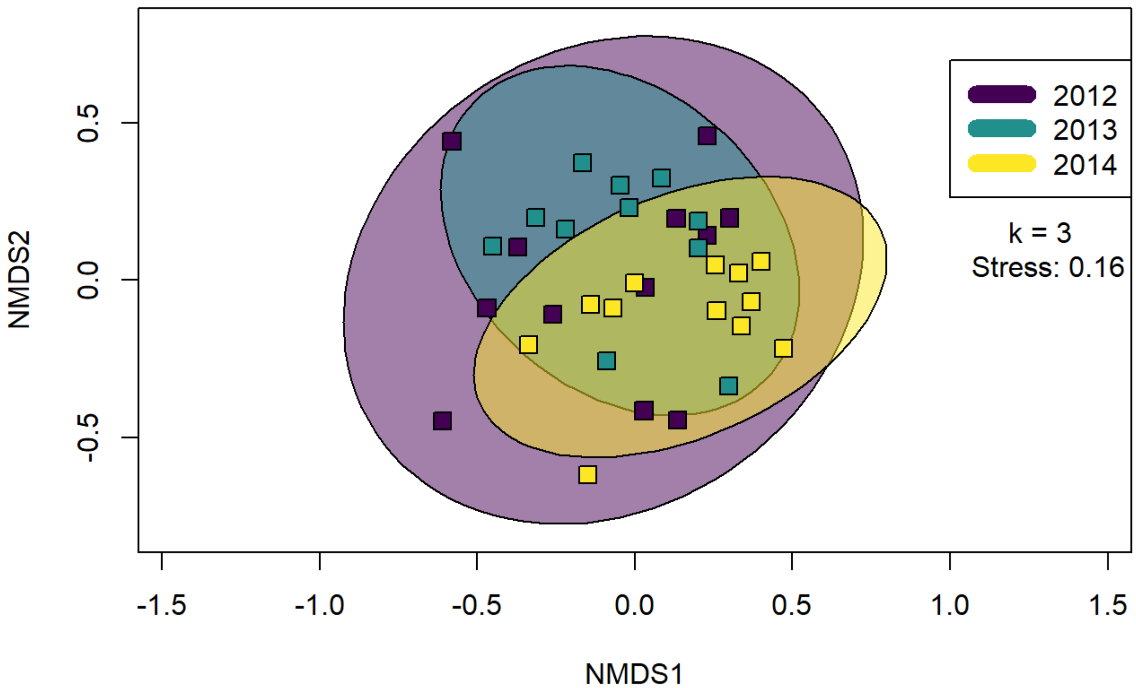

Figure 10. Non-metric MDS (four dimensions) plots found similar assemblage structures of the macrobenthic communities across all three years and all site reaches (K = 3, Stress = 0.16).

Twelve common large river metrics were selected to define interannual metric variability at each of the sample sites (quadrants scale, 400 count). A Kruskal-Wallace one-way analysis of variance was used to confirm there was no significant difference in metric values during the three years of the study (Table 9.). If significant differences ($p < 0.05$, $p < 0.01$) were observed in annual metric scores, the metric was considered not stable enough to detect trends in a three year sampling window at the given location. Knoxville had three of twelve metrics fail the assumption of no interannual differences, one with highly significant differenes, while Carderock only had two metrics fail (Table 10.). If metrics scores were not significantly different within the three year sampling window, the three years of data were compiled by site and quartile ranges defined to describe metric variability. Reported quantiles were: lower fence (inter quartile range multiplied by 1.5 and subtracted from the 25th percentile), 10th percentile, 25th percentile, 75th percentile, 90th percentile, and upper fence (inter quartile range multiplied by 1.5 and added to the 75th percentile).

Table 9. A Kruskal-Wallace one-way analysis of variance accessing metric interannual variability at each site over the three year sampling period. Non-significant results (NS) represent no differences in metric sensitivity over the sampling period. A p-value of > 0.05* represents a significant interannual change in metric and a p-value of > 0.01*** represents highly significant interannual variability.

Metric	Site	NS	<0.05	<0.01	KW p-value	KW stat
Richness	Carderock	X			0.075	5.177
	Knoxville	X			0.355	2.072
	Little Falls	X			0.358	2.055
Richness (Ephemeroptera)	Carderock	X			0.402	1.823
	Knoxville	X			0.317	2.297
	Little Falls	X			0.140	3.936
Richness (Trichoptera)	Carderock	X			0.230	2.941
	Knoxville	X			0.639	0.895
	Little Falls	X			0.255	2.736
Richness (Diptera)	Carderock *		X		0.032	6.881
	Knoxville	X			0.573	1.114
	Little Falls	X			0.176	3.472
% Chironomidae	Carderock	X			0.298	2.423
	Knoxville	X			0.232	2.926
	Little Falls	X			0.167	3.576
% Corbiculidae	Carderock	X			0.059	5.654
	Knoxville	X			0.874	0.269
	Little Falls	X			0.899	0.212
% Elmidae	Carderock	X			0.059	5.654
	Knoxville	X			0.199	3.231
	Little Falls	X			0.241	2.848
% Hydropsychidae	Carderock	X			0.211	3.115
	Knoxville ***			X	0.007	9.846
	Little Falls	X			0.654	0.848
% Ephemeroptera	Carderock	X			0.077	5.115
	Knoxville	X			0.926	0.154
	Little Falls	X			0.591	1.053
% Plecoptera	Carderock*		X		0.023	7.538
	Knoxville	X			0.944	0.115
	Little Falls	X			0.973	0.054
% Trichoptera	Carderock	X			0.981	0.038
	Knoxville*		X		0.037	6.615
	Little Falls	X			0.328	2.227
% EPT	Carderock	X			0.058	5.692
	Knoxville*		X		0.031	6.962
	Little Falls	X			0.974	0.053

Table 10. Distributions of metric values for 2012-2014, by reach.

Metric	Site	Lower Fence	10th Quartile	25th Quartile	75th Quartile	90th Quartile	Upper Fence
Richness	Carderock	18.88	25.10	26.00	30.75	33.90	37.88
	Knoxville	26.88	30.10	31.75	35.00	35.90	39.88
	Little Falls	15.00	23.00	24.00	30.00	31.00	39.00
Richness (Ephemeroptera)	Carderock	5.00	8.00	8.00	10.00	10.90	13.00
	Knoxville	7.13	8.10	9.00	10.25	11.00	12.13
	Little Falls	3.25	5.00	7.00	9.50	10.00	13.25
Richness (Trichoptera)	Carderock	3.13	5.00	5.75	7.50	9.00	10.13
	Knoxville	4.13	6.00	6.00	7.25	8.00	9.13
	Little Falls	2.75	4.00	5.00	6.50	8.00	8.75
Richness (Diptera)	Carderock *	-0.50	1.00	1.00	2.00	2.90	3.50
	Knoxville	2.00	1.10	2.00	2.00	2.00	2.00
	Little Falls	-0.50	1.00	1.00	2.00	3.00	3.50
% Chironomidae	Carderock	-3.24	0.21	0.23	2.54	3.29	6.02
	Knoxville	-1.14	0.00	0.19	1.08	3.15	2.42
	Little Falls	-0.57	0.21	0.49	1.19	3.45	2.24
% Corbiculidae	Carderock	-3.49	1.00	2.29	6.14	6.89	11.92
	Knoxville	-5.22	1.81	2.29	7.30	9.94	14.80
	Little Falls	-8.89	5.70	8.77	20.54	25.48	38.20
% Elmidae	Carderock	13.37	23.75	31.78	44.06	52.89	62.48
	Knoxville	9.41	20.84	27.43	39.44	43.01	57.46
	Little Falls	19.77	30.15	32.15	40.40	41.46	52.77
% Hydropsychidae	Carderock	5.53	12.95	14.42	20.34	21.16	29.23
	Knoxville ***	-3.29	4.24	5.83	11.92	14.30	21.04
	Little Falls	-7.06	5.96	8.06	18.14	20.69	33.26
% Ephemeroptera	Carderock	-4.91	14.52	15.55	29.18	32.88	49.64
	Knoxville	10.30	19.54	20.14	26.71	30.07	36.56
	Little Falls	-8.33	6.16	7.73	18.44	26.38	34.51
% Plecoptera	Carderock *	-2.60	0.55	1.47	4.19	5.27	8.27
	Knoxville	-0.91	0.29	0.74	1.83	2.39	3.48
	Little Falls	-3.14	0.00	0.13	2.30	4.22	5.57
% Trichoptera	Carderock	8.67	18.92	19.91	27.40	30.26	38.64
	Knoxville *	-2.76	9.01	11.84	21.57	28.72	36.16
	Little Falls	-3.03	6.81	13.62	24.72	32.10	41.37
% EPT	Carderock	24.59	34.34	44.19	57.27	63.49	76.87
	Knoxville *	21.02	33.85	37.52	48.52	53.47	65.03
	Little Falls	-0.23	20.43	27.40	45.83	52.32	73.46

Discussion and Conclusions

Mussels

Freshwater mussels are sensitive indicators of biological and water quality conditions They play a key role in large river ecology as filter feeders, removing and digesting phytoplankton and the bacteria and fungi attached to organic particles that they take in. They digest what they can of the organic material and excrete nutrients that are immediately available to plant life. They deposit unused organic material to the sediment where it becomes available for other invertebrates and fish to consume. There has been a significant collapse of freshwater mussel distributions in the United States.

All three reaches have relatively low mussel diversity, holding only four species or fewer, less than ¼ of mussel species found in the Potomac River. The Knoxville reach had the greatest mussel diversity but the lowest mussel density (Table 7). The predominance of the Eastern Elliptio (*Elliptio complanata*) at both the Carderock and Little Falls reaches is evidence of an ability to successfully colonize bedrock crevice habitats in high flow environments. These reaches are both in the Potomac Gorge which experience strong scouring flows where the river’s width is constrained resulting in flood-flow dominated habitats. The river bottoms in the Carderock and Little Falls reaches average 44.7% and 40.2%, respectively, for bedrock and boulder substrates while the Knoxville reach averages 24.1% (Table 4).

The presence of multi-year class *Elliptio complanata* collected at Carderock and Little Falls, confirmed by a large size range (from 29 mm to 108 mm in length), is evidence of successful reproduction in those reaches, an encouraging finding. A mussel survey in the Potomac undertaken in the early and mid-1980s noted the absence of young mussels and expressed concern that freshwater mussels may become extirpated from the Potomac.

River morphology and flow environments influence mussel distributions. Of the four mussel taxa observed in this study, all are considered “generalists” in habitat preference and flow tolerance. *Alasmidonta*, *Elliptio*, *Lampsilis*, and *Strophitus* establish in a wide variety of flow regimes, requiring only minimal habitat availability to colonize. High flows will reorganize the distribution of finer particle substrate sizes (Benda et al. 2004), and thus may affect mussel habitat availability as well as mussel distributions. Each of the mussel taxa in this study was observed in both highly mobile sand and gravel habitats as well as in fissures within the boulder/bedrock substrates. The ability of these mussel taxa to colonize in both high-flow/high-disturbance habitats and in low flow/low disturbance habitats shows the plasticity and robustness of the mainstem mussel species.

The ability of these freshwater mussels to opportunistically occupy many different macrohabitats leads to high variation in detection limits and density calculations due to clustered mussel distributions and variable detection ability within each habitat. For example, the CPUE (mussels per hour) for individuals at sites more fortified with bedrock substrate (Carderock and Little Falls) was significantly higher than the Knoxville site, a wider slower reach. This can be explained in several ways. The bedrock substrate of the Carderock and Little Falls sites provide only marginal habitat in the form of small debris eddies and bedrock fissures for mussel settlement.

The ability to rapidly quantify clustered assemblages of mussels in a habitat bound by bedrock which provides little cover leads to a much higher collection efficiency. Conversely, the Knoxville site is described as having more fine particle habitat that is not bound by bedrock. Without bedrock morphology bounding the distribution ability of the mussels, they are able to settle in a less clustered distribution in an environment that additionally provides greater cover.

The inclusion of mussels as part of this large river ecological study, while desirable, required substantial staff time and effort to access and sample individual reaches. Often at least one full day with a crew of at least two biologists was required to adequately sample each of the three reaches. Although this effort was intensive, documentation of freshwater mussel distribution was especially important in the Carderock and Little Falls reaches as they have historically been understudied despite an environmental flow-by recommendation of 300 mgd at Carderock and an environmental flow-by requirement of 100 mgd at Little Falls. The findings of this study should be helpful to agencies and stakeholders researching the impacts of prolonged drought or extreme high flow events.

Benthic Macroinvertebrates

Benthic macroinvertebrates represent a fundamental link in the food web between terrestrial and instream organic matter sources (e.g., leaf packs, periphyton, detritus) and vertebrates such as reptiles, amphibians, and fishes. When biological range distributions and biogeography can be accounted for, riverine macroinvertebrate communities respond in predictable ways to changes in environmental conditions. Macroinvertebrates are particularly well suited for assessing site-specific stressors because of their limited ability to migrate or move between river systems, and their dependence on specific water quality parameters during multiple life stages.

In the early 1970s, at ICPRB's urging, the Maryland Water Resources Administration initiated a Potomac Baseline Water Quality Monitoring Network which evaluated the benthic macroinvertebrates collected with multi-plate and Surber samplers at mainstem stations from the North Branch to Little Falls (ICPRB 1978). They reported finding "fair" to "excellent" macroinvertebrate communities at Point of Rocks, the closest site to our Knoxville reach (9 miles downstream), but "poor" and "stressed" communities at Little Falls. Their studies did not include a site representative of Carderock.

The Maryland Power Plant and Environmental Review's "Long-Term Benthic Monitoring Studies in the Freshwater Portion of the Potomac River –1983-1985" used a scuba deployed "Benthic Dome Sampler" that collected a 0.16 m² area in predominantly soft substrate up to 4 m deep. The report's Station #7 was located just upstream from the Monocacy River confluence and was the closest station to our Knoxville reach. There were no stations from Great Falls to Little Falls. They reported an average depth of 0.85 m, which is not far from the average depth in this study of 0.69 m. Also of interest, they noted *Ephoron leukon*, the White Miller mayfly, was one of their key species. *Ephoron leukon* was not found in our study, perhaps due to differences in methodology as their grab samplers were deployed primarily in silts, a preferred habitat of the species. Despite differences in sampling methodology, one would not expect the White Miller to be absent in our samples when the preferred habitat. sand and silt, were approximately 18% of our sampled area. Long term Potomac anglers report that they have noticed a great reduction in the numbers of whitefly hatches which they say were seasonally common during the 1980s and 1990s (pers. comm. to J. Cummins, as well as personal observation in the 1990s).

Multi-plate sample results from the Maryland Core/Trend Program indicate the macroinvertebrate community was in “Fair/Good” condition at their Little Falls station (POT1183, #61 in Figure 1) according to MD-DNR rating guidelines and showed no change between 1981 and 2004 (Friedman 2009). Macroinvertebrate condition at the program’s Whites Ferry station (POT1471, #62 in Figure 1), between this study’s Carderock and Knoxville reaches, was solidly “Good” and showed slight improvement between 1976 and 2004 due to increasing *Ephemeroptera* taxa. Macroinvertebrate condition at the program’s Point of Rocks (POT1595, #73 in Figure 1) station, downstream of this study’s Knoxville reach, was also solidly “Good” and showed no overall change between 1976 and 2006. Composition was changing, however, with % Trichoptera (*Hydropsychidae*) decreasing, and the number of EPT taxa and % *Stenochironomus* sp. (pollution sensitive Diptera) increasing. This may reflect a shift in food substrate from (planktonic) algae to living and dead aquatic vegetation (Friedman 2009).

Macroinvertebrate samples were collected in 2008 by the U. S. National River and Stream Assessment program (https://www.epa.gov/national-aquatic-resource-surveys/nrsa) at three sites between Point of Rocks and this study’s Carderock reach. Samples were collected using a D-frame kick net with a 500 µm mesh, the same method used in our study. A sample count of 300 organisms was made to the lowest taxonomic level. Values of the macroinvertebrate metrics used in assessing the site are shown in Table 11. The three sites each received a rating of “Fair” according to the national guideline established for NRSA sites.

Table 11. Metrics calculated from macroinvertebrate data collected for the 2008 U. S. National River and Stream Assessment survey from three sites on the lower Potomac River mainstem.

Macroinvertebrate Metric	Upstream of Seneca Creek (UID = 11477)	Downstream of Mason Island (UID = 11479)	Upstream of Mason Island (UID = 11485)
% Burrower	23.08	21.43	20.51
% Ephemeroptera	17.95	16.67	17.95
# Ephemeroptera Taxa	10	12	12
Shannon Diversity	2.49	2.91	2.75
# Scrapper Taxa	7	9	8
# Tolerant Taxa (PTV≥7)	20.51	19.05	23.08

One goal of ICPRB’s Large River Study 2012-2014 was to investigate the US EPA’s National Rivers and Streams Assessment and Maryland’s Core/Trend methodologies for assessing biological condition of the lower Potomac River. The field methods used in these two programs are summarized in Appendix 6. We knew the results from our study using the benthic-kick protocol would probably not be comparable to results of the MD Core Trend which uses Serber and Hester-Dendy plates, a passive sampling methodology that is well known to differ from kick net methods (Guild et al. 2014).

ICPRB's single habitat assessment technique yielded results similar to those of NRSA for 2008. Although both methodologies arrived at similar metric scores, we believe two methodological are somewhat different. First, NRSA processes samples at the 300-count level. The 300-count processing effort is more than adequate for percent metrics but may miss an opportunity to optimize effort/cost for richness metrics. Richness metric variability drops from ~8% to below 5% accuracy with an increase from 300-count to 400-count samples (Figure 9). Second, the NRSA protocols implement a proportional multihabitat approach for wide spread application across the United States. In many Central and Western US streams, woody debris and other complex structures are frequently observed and make up a significant portion of the available in-stream habitat. This is not the case for the lower Potomac which is lined with bedrock not conducive to the establishment of woody complex structures. For this reason, a proportional macrohabitat methodology ultimately converges on results similar to the single habitat approach due to the more homogenous habitat in the Lower Potomac River. Due to the habitat composition of the lower Potomac River, single habitat and multi-habitat active sampling procedures are considered, with caution, to be comparable for future trend work until an adequate baseline and procedure is defined.

This study's results confirm that macroinvertebrate communities at Knoxville, Carderock, and Little Falls are, at present, very similar in moderate flow years. Distributions of the values of several macroinvertebrate metrics by year and location are provided in Appendix 7.

Macrophytes

It was determined at the end of the three-year study that random ¼ m^2 quadrats are not sufficient, both logistically and statistically, to evaluate the spatial density and distribution of aquatic vegetation in the Potomac River mainstem. Submerged aquatic vegetation, filamentous green algae, benthic filamentous blue green algae, and periphyton are all susceptible to fine scale flow regimes and manifest in clustered distributions. The clustered distributions and scale of established vegetation cannot be properly captured in small, randomly sampled grid cells. Future efforts to capture aquatic vegetation distributions in the mainstem Potomac River should include aerial surveys of vegetative cover followed by professional identifications in captured areas.

Impacts of Extreme Flows

The Potomac River enters a fall zone called Mather Gorge as it approaches Carderock and Little Falls. The gorge is an area of special concern because of its unique and rare biological communities (Cummins et. al. 2010). The river in the gorge is bound by steep bedrock banks and ledges and has no flood plain to dissipate flow energy. Intensifying floods related to climate change and prolonged droughts exacerbated by large consumptive uses and metropolitan Washington water supply withdrawals have an increased potential to disrupt mussel and macroinvertebrate communities in the Gorge.

One objective of this study was to contribute a baseline dataset for evaluating future flow impacts on the freshwater mussels and benthic macroinvertebrates in the lower free-flowing Potomac River segment, and particularly the Gorge. Annual flows during the 3-year study period classified as moderate according to Olson's Flow Index and did not experience extreme flooding or severe drought. The flow metric values reported in Table 6 and Figure 6 can serve as benchmarks of a moderate hydrological year for this river segment. In the future, these same metrics can be used to characterize flow during prolonged drought or very wet periods. The corresponding changes in macroinvertebrate and mussel communities exposed to these extreme conditions can then be compared to the communities documented in this study.

Information Gaps and Future Research

The mainstem Potomac River is a dynamic, changing system that experiences natural and anthropogenic stressors to which its biological communities are forced to respond. The Maryland Core Trend Monitoring Program has maintained six long-term sites on the 170 mile Potomac River mainstem between the confluence of the river's North Branch and South Branches and its tidal estuary, and its results are used to determine trends in four macroinvertebrate metrics (e.g., Friedman 2009). Shorter-term sampling efforts, such as the Maryland Power Plant study, USGS National Rivers and Streams Assessment, and Potomac Basin Large River Environmental Flow Needs, have collected data at other mainstem sites but lacked a common methodology for making direct comparisons and detecting trends. ICPRB performed this study to provide taxa lists, catch per unit effort (CPUE), and metric variability information for moderate flow years in the recent period. The intent is to aid in future agency program designs and promote methodologies that are relatable across time and space. Continued investigations are needed to develop appropriate large river biological metrics and methodologies, and identify the associated scale and effort required to improve the accuracy and precision of the metrics. As a follow up to this report, ICPRB will investigate within-site benthic macroinvertebrate variability due to flow and influence of parent tributary.

The Potomac River mainstem does not yet have designated reference reaches, metrics, or indices with which to compare our findings. This study therefore cannot state, nor intends to state, whether the Lower Potomac River is in Good, Fair, or Poor condition. Due to the inconsistency of data collection methodologies and consideration of the synergistic effects of biological, land-use, hydrological, and chemical variables critical to describing river health, this report intends to act as a starting point for long term trend analyses in the Potomac mainstem using the more widely used kick net method. Since a single habitat method was used in this study, direct comparisons to this study should utilize a single habitat approach in the future. Until a unified approach is determined, comparability between multihabitat and single habitat approaches yield similar results due to the lack of complex structures (woody debris, detritus etc.) in the lower Potomac River and therefore may cautiously be considered comparable to a single habitat method in this region.

ICPRB will continue to sample the large rivers of the Potomac River Drainage as flow conditions allow. During the duration of this study, flow conditions remained within average levels, and so, did not allow for any investigation into high flow and drought years. Ideally drought and flood conditions are needed to identify changes in community structure from environmental stress, the three-year time span of this study was able to successfully develop a robust baseline dataset that can be used as a point of reference in the future. ICPRB intends to extend its large river efforts to more reaches along the length of the entire Potomac River mainstem in an attempt to document regional as well as seasonal differences in the Potomac drainage. Additionally, ICPRB biologists hope to share the methodologies and results from this study and apply them to other large river drainages, such as the Susquehanna and James river systems.

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Appendices

Appendix 1: Sample Grids for Little Falls, Carderock, and Knoxville Reaches

Appendix 2: Field Form for Mainstem Freshwater Mussels

Appendix 3: Macroinvertebrate Data Preparation Steps

Appendix 4: SAV / Filamentous Algae / Periphyton Field Form

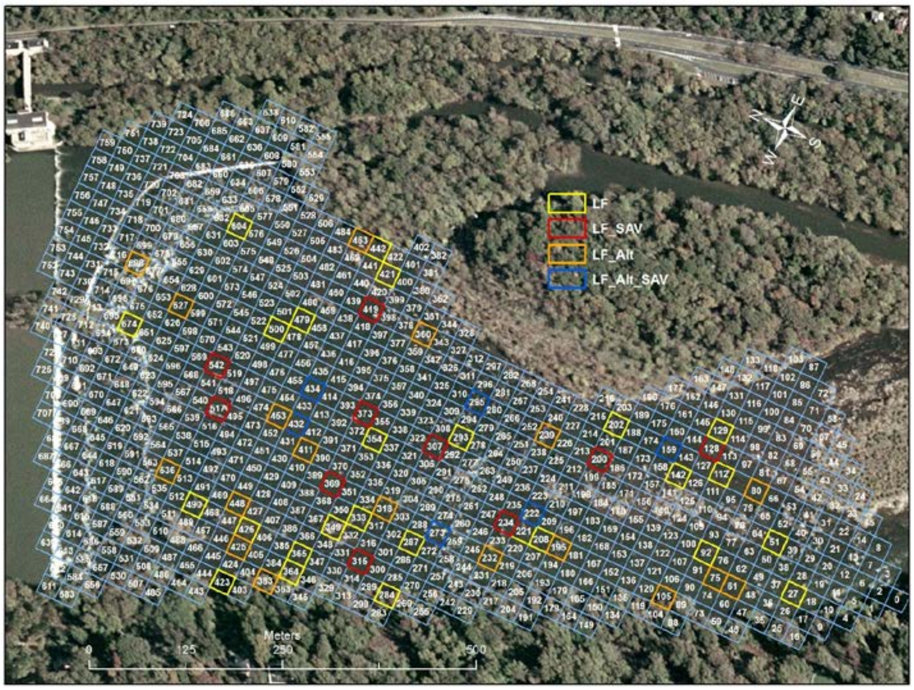
Appendix 5: Ranked Macroinvertebrate Taxa Lists, by Year and Reach

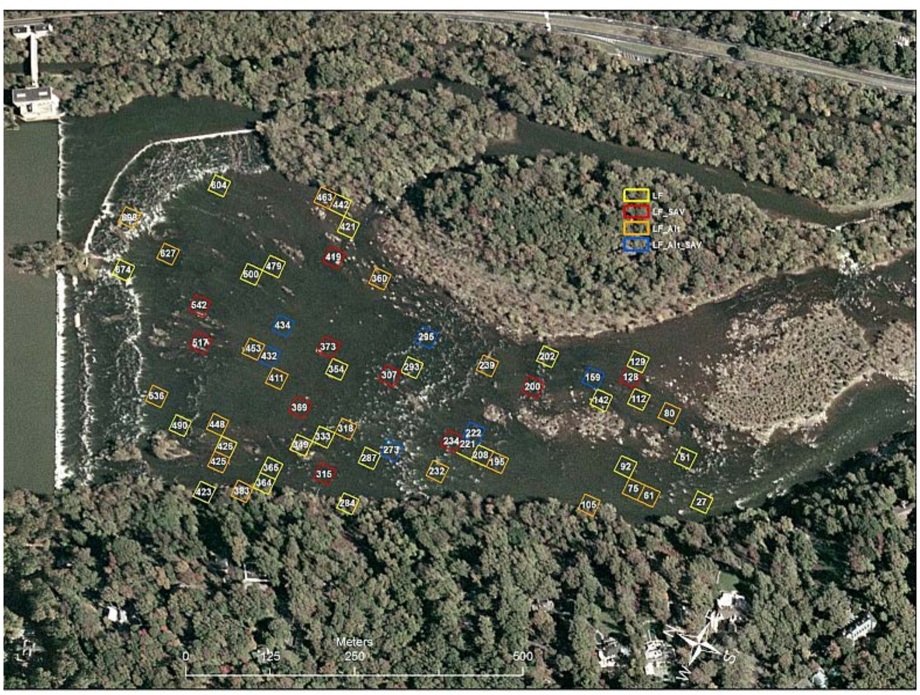
Appendix 6: EPA and MDDNR Monitoring Program Methods

Appendix 7. Box Plots of Large River Metric Variability

Appendix 1: Sample Grid Example for Little Falls

Grid layout at Little Falls reach. Yellow boxes are primary sample sites, red boxes are primary sample sites where submerged aquatic vegetation transects were conducted, orange and blue reaches are alternate reaches.





Appendix 2: Field Form for Mainstem Freshwater Mussels

River Reach ____________________________ Section__________ Date:_____/____/______
StartTime ___:___ Air Temp ____ Water Temp _____ D.O. ____ Cond. ________ pH _____
Water Clarity (least is .5 m, then in 0.25 meter increments)_______Weather_________________

Site #	Substrate (Est %) --------------------- Habitat Type (Circle)	Depth and Est. % Avl* Habitat	SAV Type & % Cover %FGA/BGA	Time Quad (Vis, Exc) Vicinity (2m Dia)	Detects Species, Sizes (L-D-W, in mm) and number detected Dead mussel shells measured for L, Denote** Fresh, Subfossil, Fossil	Notes
	Bed= Bol = Cob= Gra = San = Silt = Oth = ---------------------- Po Gl Ri Ra Ca Fa Other:	Water Depth. In .1 m __.__ Est. % Avl Hab. 1) Quad ____% 2) Vic ____%	Quad: Vic: FGA BGA	Vis = ___:___ Exc = ___:___ Vic = ___:___	Vis = Exc = Est. # Corbs =____ Vic =	Current***: Still - Lite - Mod - Strong Sunlight: Open or % Overstory
	Bed= Bol = Cob= Gra = San = Silt = Oth = ---------------------- Po Gl Ri Ra Ca Fa Other:	Water Depth. In .1 m __.__ Est. % Avl Hab. 1) Quad ____% 2) Vic ____%	Quad Vic FGA BGA	Vis = ___:___ Exc = ___:___ Vic = ___:___	Vis = Exc = Est. # Corbs =____ Vic =	Current: Still - Lite - Mod – Strong Sunlight: Open or % Overstory
	Bed= Bol = Cob= Gra = San = Silt = Oth = --------------------- Po Gl Ri Ra Ca Fa Other:	Water Depth. In .1 m __.__ Est. % Avl Hab. 1) Quad ____% 2) Vic ____%	Quad Vic FGA: BGA:	Vis = ___:___ Exc = ___:___ Vic = ___:___	Vis = Exc = Est. # Corbs =____ Vic =	Current: Still - Lite - Mod – Strong Sunlight: Open or % Overstory

Surveyor(s):______________________________Gage (_______)
Flow/Median______/_______

* Estimates for both Quad and Site - includes all small substrate (silt to cobble, "other" as judged appropriate, such as "shells") plus crevice areas of boulders and bedrock.
** Fresh = shell has bits of internal tissue, Subfossil = no internal tissue, nacre still lustrous, most of peristracum is present, Fossil = no internal tissue, nacre dull, most peristracum is gone,
***Still = no discernable current, Lite = discernable but easy to stay in place, Mod = requires swimming to stay in place, Strong = requires anchorage to stay in place.

Appendix 3. Macroinvertebrate Data Preparation Steps

Taxa preparation R-scripts for metric analysis. R-coding defines when a taxanomic group needs to be rounded up or adjust for updated naming from the ITIS database.

```
Taxa Prep:
genus = case_when(
family == "chironomidae" ~ "chironomidae",
family == "pisidiidae" ~ "pisidiidae",
class == "oligochaeta" ~ "oligochaeta",
phylum == "platyhelminthes" ~ "platyhelminthes",
final_id == "serratella_deficiens" ~ "teloganopsis",
final_id == "tvetenia_discoloripes" ~ "chironomidae",
final_id == "sphaeriidae" ~ "pisidiidae",
final_id == "turbellaria"  ~ "platyhelminthes",
final_id == "tubificidae"  ~ "oligochaeta", TRUE ~ genus)
```

Appendix 4: SAV / Filamentous Algae / Periphyton Field Form

River Reach ________________________________ Section______ Site #________

Date: ____/____/201_ Surveyor: _________________Recorder:_____________________

Note: Indicate **RL** for river-left or **RR** for river-right in the left margin at the respective start points.

Length in 0.10 m.	Depth in 0.10m	SAV/Algae Species*	Periphyton/Sediment Cover** **0**None/**1**Light/**2**Med/**3**Heavy	Predominant substrate **Be**d, **Bo**l,**Co**b,Gra, **Sa**n, **Si**l, **Ot**h
[**RL**] 0 -				

*See back for ID tips and abbreviations. **is estimated % coverage on leaf surface, 0 = 0-10%, 1 = 10-30%, 2 = 30-50%, 3 = >

Appendix 4 continued, the back page of the SAV/Algae/Periphyton Field Form.

Identification Tips*	Scientific Name	Common Name	Abbr.
Submerged Grasses			
Basal Leaves =	Vallisneria Americana	Water Celery	VAL
Whorled Leaves			
Simple leaf			
5-leaved =	Hydrilla verticillata	Hydrilla	HY
3-leaved =	Elodia spp.	Elodea	El
4-8 larger leaves, thick stem	Egeria densa	Brazilian Weed, Anacharis	BW
Compound leaf			
Roughly divided, 9-10 leaves, stiff =	Ceratophyllum demersum	Coontail	CT
Finely divided, loose			
5-leaved whorls =	Myriophyllum brasiliense	Parrot Feather	PF
4-leaved whorls =	Myriophyllum spicatum	Eurasian Milfoil	EM
Opposite Leaves			
Leaf tip angle $<90^\circ$			
Most leaves > 4 cm long =	Zannichellia palustris	Horned Pondweed	HP
All leaves < 4 cm long =		Naiad spp.	NSpp
Flattened Leaves, no teeth =	Najas quadalupensis	Southern Naiad	SON
Recurved Leaves, strongly toothed =	N. minor	Spiny Naiad	SPN
Fine, "straight" Leaves, weakly toothed			
Very fine wavy leaves =	N. flexilis	Northern Naiad	NON
Very fine straight leaves =	N. gracilliama	Slender Naiad	SLN
Leaf tip angle $>90^\circ$, floating egg-shaped upper leaves =	Callitriche spp.	Water Starwort	WS
Alternate Leaves			
Leaves < 2 mm wide			
Visible midrib =	Potamogeton pusillus	Slender Pondweed	SLP
No prominent midrib			
All leaves > .5 mm wide =	Stuckenia pectinata	Sago Pondweed	SAP
All leaves < .5 mm wide =	Ruppia maritime	Widgeon grass	WG
Leaves >2mm wide			
Prominent midrib			
Wavy Leaves =	Potamogeton crispus	Curly Pondweed	CUP
Leaves wrap around stem =	Potamogeton perfoliatus	Redhead Grass	RG
No prominent midrib =	Heteranthra dubia	Water Stargrass	SG
Emergent Grass =	Justicia americana	Water Willow	WW
Floating Grass = Triangular or diamond-shaped leaves	Trapa natans	Water Chestnut	ACK
Algae			
Brittle, skunky smelling, whorled-like axis	Chara spp.	Muskgrass	CH

Compiled from: "Underwater Grasses in the Chesapeake Bay & Mid-Atlantic Coastal Waters"

Appendix 5: Ranked Macroinvertebrate Taxa Lists, by Year and Reach

Knoxville reach. Accumulative and inter-annual counts of benthic taxa collected at fixed sampling station, Knoxville during 2012 to 2014. Sorted by Rank.

Rank	Taxon	Count	2012	2013	2014
1	Stenelmis	1555	594	377	584
2	Isonychia	388	90	144	154
3	Cheumatopsyche	364	32	196	136
4	Tricorythodes	292	111	90	91
5	Corbicula	282	65	77	140
6	Gammarus	272	75	46	151
7	Anthopotamus	173	95	17	61
8	Baetis	135	36	46	53
9	Helicopsyche	124	47	75	2
10	Hydropsyche	123	30	68	25
11	Corydalus	114	38	17	59
12	Argia	102	55	15	32
13	Leptoxis	95	42	36	17
14	Chimarra	93	21	12	60
15	Chironomidae	84	8	17	59
16	Psephenus	59	36	6	17
17	Cnephia	56	6	50	0
18	Leucrocuta	55	9	19	27
19	Orthotrichia	53	0	53	0
20	Heterocloeon	52	3	27	22
21	Maccaffertium	50	5	18	27
22	Optioservus	48	15	17	16
23	Protoptila	48	0	0	48
24	Agnetina	45	24	6	15
25	Oligochaeta	34	7	11	16
26	Teloganopsis	26	8	8	10
27	Acroneuria	24	6	10	8
28	Simulium	23	14	0	9
29	Neureclipsis	20	4	6	10
30	Macrostemum	19	5	8	6
31	Microcylloepus	14	0	2	12
32	Baetidae	13	9	1	3
33	Glossosomatidae	12	12	0	0
34	Petrophila	10	2	3	5
35	Plauditus	10	3	1	6
36	Sialis	9	3	1	5
37	Bithynia	8	0	8	0
38	Caenis	8	1	0	7
39	Platyhelminthes	7	2	1	4
40	Acentrella	6	2	4	0

Knoxville reach (continued). Accumulative and inter-annual counts of benthic taxa collected at fixed sampling station, Knoxville during 2012 to 2014. Sorted by Rank.

Rank	Taxon	Count	2012	2013	2014
41	Heptageniidae	6	0	6	0
42	Pisidiidae	6	1	3	2
43	Hydroptila	5	0	3	2
44	Procloeon	5	5	0	0
45	Serratella	5	0	5	0
46	Agapetus	4	4	0	0
47	Amnicola	4	0	4	0
48	Dubiraphia	4	1	2	1
49	Gomphus	4	0	2	2
50	Macronychus	4	2	0	2
51	Ectopria	3	2	0	1
52	Hetaerina	3	1	1	1
53	Physa	3	1	2	0
54	Pseudocloeon	3	2	0	1
55	Acerpenna	2	1	1	0
56	Elmidae	2	2	0	0
57	Ephemeroptera	2	0	2	0
58	Gomphidae	2	0	0	2
59	Hirudinea	2	1	1	0
60	Hydroptilidae	2	0	1	1
61	Neoperla	2	0	2	0
62	Ochrotrichia	2	0	2	0
63	Pycnopsyche	2	2	0	0
64	Antocha	1	0	0	1
65	Berosus	1	0	1	0
66	Calopteryx	1	1	0	0
67	Ceratopsyche	1	0	1	0
68	Crambidae	1	1	0	0
69	Dromogomphus	1	1	0	0
70	Elliptio	1	0	1	0
71	Ephemerellidae	1	1	0	0
72	Erpobdella	1	0	0	1
73	Glossiphoniidae	1	1	0	0
74	Glossosoma	1	1	0	0
75	Hansonoperla	1	0	1	0
76	Helichus	1	1	0	0
77	Helisoma	1	0	1	0
78	Hemerodromia	1	0	1	0
79	Hydrobiidae	1	0	0	1
80	Lepidostoma	1	0	0	1

Knoxville reach (continued). Accumulative and inter-annual counts of benthic taxa collected at fixed sampling station, Knoxville during 2012 to 2014. Sorted by Rank.

Rank	Taxon	Count	2012	2013	2014
81	Leptoceridae	1	0	0	1
82	Limnephilidae	1	0	0	1
83	Oravelia	1	0	1	0
84	Perlinella	1	0	0	1
85	Sisyridae	1	0	0	1
86	Stenacron	1	1	0	0
87	Trichoptera	1	1	0	0

Carderock reach. Accumulative and inter-annual counts of benthic taxa collected at fixed sampling station, Carderock during 2012 to 2014. Sorted by Rank.

Rank	Taxon	Count	2012	2013	2014
1	Stenelmis	1871	456	547	868
2	Cheumatopsyche	580	188	157	235
3	Baetis	291	46	178	67
4	Corbicula	200	84	27	89
5	Hydropsyche	173	48	79	46
6	Anthopotamus	172	71	42	59
7	Isonychia	149	112	22	15
8	Maccaffertium	143	71	47	25
9	Heterocloeon	138	10	51	77
10	Brachycentrus	131	60	67	4
11	Agnetina	118	61	45	12
12	Macrostemum	117	46	11	60
13	Tricorythodes	92	10	60	22
14	Chironomidae	79	39	29	11
15	Leucrocuta	76	7	67	2
16	Protoptila	76	0	0	76
17	Petrophila	67	33	26	8
18	Orthotrichia	59	0	59	0
19	Plauditus	53	40	7	6
20	Leptoxis	52	21	28	3
21	Gammarus	42	26	0	16
22	Oligochaeta	41	9	10	22
23	Neoperla	38	21	14	3
24	Chimarra	36	5	4	27
25	Neureclipsis	29	27	2	0
26	Optioservus	29	10	6	13
27	Corydalus	24	7	8	9
28	Argia	20	18	2	0
29	Cnephia	16	0	16	0
30	Teloganopsis	15	1	12	2
31	Stactobiella	13	0	13	0
32	Hemerodromia	12	0	4	8
33	Acentrella	10	5	5	0
34	Psephenus	8	2	3	3
35	Heptageniidae	7	5	0	2
36	Platyhelminthes	7	3	3	1
37	Baetidae	6	1	5	0
38	Helicopsyche	6	3	3	0
39	Simulium	6	0	0	6
40	Macronychus	5	5	0	0

Carderock reach (continued). Accumulative and interannual counts of benthic taxa collected at fixed sampling station, Carderock during 2012 to 2014. Sorted by Rank.

Rank	Taxon	Count	2012	2013	2014
41	Pleurocera	5	5	0	0
42	Ancylidae	4	4	0	0
43	Goniobasis	4	0	4	0
44	Stenacron	4	4	0	0
45	Acerpenna	3	3	0	0
46	Amnicola	3	3	0	0
47	Physa	3	3	0	0
48	Acroneuria	2	1	1	0
49	Ceraclea	2	1	1	0
50	Hydropsychidae	2	2	0	0
51	Hydroptila	2	1	1	0
52	Hydroptilidae	2	1	1	0
53	Microcylloepus	2	0	0	2
54	Taeniopteryx	2	2	0	0
55	Bithynia	1	0	1	0
56	Caenis	1	0	0	1
57	Coenagrionidae	1	1	0	0
58	Dubiraphia	1	0	1	0
59	Ectopria	1	1	0	0
60	Ephemerellidae	1	0	0	1
61	Ephemeroptera	1	0	1	0
62	Gomphus	1	0	1	0
63	Hagenius	1	0	0	1
64	Lepidostoma	1	0	0	1
65	Leptoceridae	1	0	0	1
66	Lymnaeidae	1	1	0	0
67	Macromia	1	1	0	0
68	Mystacides	1	1	0	0
69	Neurocordulia	1	1	0	0
70	Oecetis	1	0	0	1
71	Polycentropus	1	1	0	0
72	Serratella	1	1	0	0

Little Falls reach. Accumulative and interannual counts of benthic taxa collected at fixed sampling station, Little Falls during 2012 to 2014. Sorted by Rank
.

Rank	Taxon	Count	2012	2013	2014
1	Stenelmis	1446	551	295	600
2	Corbicula	588	185	173	230
3	Cheumatopsyche	259	25	100	134
4	Macrostemum	182	90	43	49
5	Baetis	174	48	15	111
6	Chimarra	169	38	61	70
7	Hydropsyche	169	45	81	43
8	Gammarus	159	13	22	124
9	Heterocloeon	104	41	17	46
10	Tricorythodes	81	45	12	24
11	Brachycentrus	77	42	23	12
12	Argia	73	35	30	8
13	Chironomidae	71	55	10	6
14	Isonychia	68	16	50	2
15	Agnetina	58	5	27	26
16	Anthopotamus	45	27	2	16
17	Leptoxis	43	24	16	3
18	Oligochaeta	29	8	8	13
19	Teloganopsis	29	7	3	19
20	Petrophila	26	11	9	6
21	Maccaffertium	24	5	10	9
22	Corydalus	23	10	4	9
23	Bithynia	21	9	9	3
24	Leucrocuta	20	12	2	6
25	Neoperla	18	15	2	1
26	Microcylloepus	16	11	1	4
27	Plauditus	16	2	0	14
28	Cnephia	13	1	12	0
29	Psephenus	10	3	3	4
30	Helicopsyche	9	0	9	0
31	Baetidae	7	2	0	5
32	Acentrella	6	6	0	0
33	Heptageniidae	6	5	0	1
34	Platyhelminthes	6	1	1	4
35	Protoptila	6	0	0	6
36	Hydrobiidae	5	5	0	0
37	Potamyia	5	0	0	5
38	Simulium	5	0	1	4
39	Procloeon	4	4	0	0
40	Trichoptera	4	0	4	0

Little Falls reach (continued). Accumulative and interannual counts of benthic taxa collected at fixed sampling station, Little Falls during 2012 to 2014. Sorted by Rank.

Rank	Taxon	Count	2012	2013	2014
41	Elliptio	3	2	0	1
42	Erpobdella	3	0	2	1
43	Hirudinea	3	3	0	0
44	Hydropsychidae	3	0	3	0
45	Hydroptilidae	3	2	0	1
46	Neureclipsis	3	1	1	1
47	Optioservus	3	2	0	1
48	Acroneuria	2	0	2	0
49	Empididae	2	0	0	2
50	Sialis	2	1	1	0
51	Anisoptera	1	0	1	0
52	Caenis	1	0	0	1
53	Ceraclea	1	1	0	0
54	Corydalidae	1	0	0	1
55	Elimia	1	0	0	1
56	Ephemerellidae	1	0	0	1
57	Ephemeroptera	1	0	1	0
58	Gastropoda	1	1	0	0
59	Gomphus	1	1	0	0
60	Goniobasis	1	0	1	0
61	Hetaerina	1	1	0	0
62	Lirceus	1	1	0	0
63	Nematoda	1	0	0	1
64	Nigronia	1	1	0	0
65	Oecetis	1	0	0	1
66	Pleurocera	1	1	0	0

Appendix 6: EPA and MDDNR Monitoring Program Methods

Environmental Protection Agency: National Rivers and Streams Assessment

The National Rivers and Streams Assessment (NRSA) is a national probability-based survey of rivers and streams based on physical, chemical and biological data collected and analyzed using standardized field and laboratory methods. NRSA was designed to determine the extent to which rivers and streams support a healthy biological condition and the extent of major stressors that affect them. The survey supports a longer-term goal: to determine whether our rivers and streams are getting cleaner and how we might best invest in protecting and restoring them. Additionally, the survey compares the condition of streams to those of an earlier study that focused on small streams (the Wadeable Streams Assessment or WSA) conducted by the U.S. Environmental Protection Agency and its partners in 2004. Benthic macroinvertebrate data were collected at each study site over a length of 40 times the channel width, with a minimum reach length of 150 m and a maximum reach length of 4 km (USEPA 2007). Data were collected using two different sampling methods, with the first being the standard reachwide method (RW) used to collect data supporting the WSA (USEPA 2004), and the second being the alternate LG method (USEPA 2007). For each method, a 0.09 m^2 quadrat sample was collected at each of 11 transects equally distributed along the same sample reach (D-frame net: 500 μm mesh). Samples were alternately collected at either a left, center, or right point along each transect with the initial location on the first transect being randomly selected (USEPA 2007). For the RW method, collection points were located at 25, 50, or 75 % of the stream width; common habitats sampled included bottom substrate, woody debris, macrophytes, and leaf packs. For the LG method, collection points were located at 0, 50, or 100 % of the stream width; this method included stream edge habitats (e.g., undercut banks and root wads) that the RW method frequently did not. The LG method's inclusion of edge habitats also likely increased the frequency of sampling snags and macrophyte beds. The initial location for the RW sample was randomly selected. Then, the LG sample was shifted to the next location in a left, center, or right configuration. All subsequent transects were shifted one position or location to the "right" until all 11 transects were sampled.

For each method, collected samples were composited and field preserved with ~95% ethanol. In the laboratory, with the goal of having 300 organisms available for identification, a randomized 500-organism subsample was sorted using a gridded screen and preserved separately from the rest of the sample (USEPA 2008). If a sample contained fewer than 300 organisms, the entire sample was sorted. Sorted organisms were then identified to the taxonomic level specified for the study (usually genus; USEPA 2008). Field methods for all parameters are described in detail in the NRSA field manual (USEPA 2007).

Maryland Dept. of Natural Resources/ Maryland Dept. of Energy Core/Trend Monitoring Program

Maryland's Ambient Water Quality Monitoring Program (Core/Trend Monitoring) is part of a nationwide ambient monitoring effort designed to measure progress towards achieving EPA's national water quality goals. This program was initiated in 1974 to meet an EPA-mandated monitoring requirement for Maryland to collect data that can be used to detect status and trends in the quality of the state's waters. The program was initially implemented by Maryland Department of the Environment, and transferred to Maryland Department of Natural Resources as of 1 July 1995.

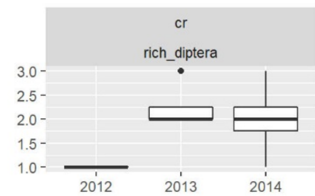
A network of fixed stations located in most of the state's larger, non-tidal streams and rivers of Strahler 4th order and larger are routinely sampled for the Program (Report Figure 2). Biological and water quality samples collected in the Core/Trends network are used to assess status and also examine long-term trends.

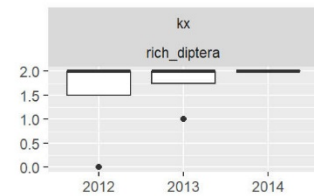
Benthic macroinvertebrate assemblages are sampled annually at a subset of stations using Surber and Hester-Dendy multiplate samplers. Samples are collected and processed by MDDNR's Monitoring and Non-Tidal Assessment staff. Six monitoring stations are located in the mainstem Potomac River (Report Figure 2).

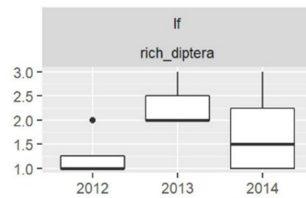
Appendix 7: Boxplots of Large River Metric Variability

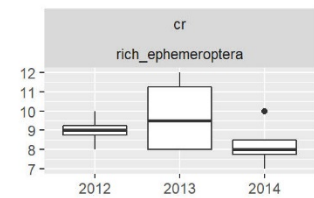
Locations: cr, Carderock; kx, Knoxville; lf, Little Falls.

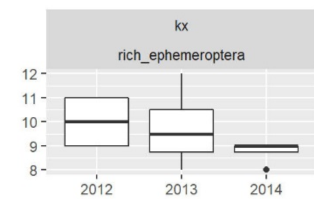
400 Count Metrics: rich_diptera, number of Diptera in a count sample; rich_ephemeroptera, number of Emphemeroptera in a sample; rich_trichoptera, number of Trichoptera in a sample; rich, taxa richness in a sample; pct_chironomidae, percent of total count as Chironomidae; pct_elmidae, percent of total count as Elmidae; pct_ephemeroptera, percent of total count as Ephemeroptera; pct_ept, percent of total count as Ephemeroptera, Plecoptera, and Trichoptera; pct_hydropsychidae; percent of total count as Hydropsychidae; and pct_plecoptera, percent of total count as Plecoptera.

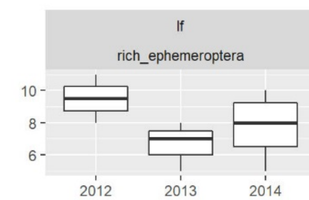


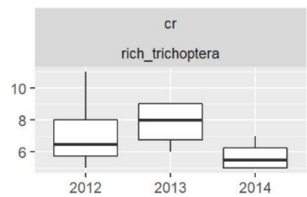








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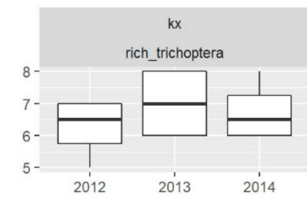
10
8
6
2012
2013
2014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7
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5
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2013
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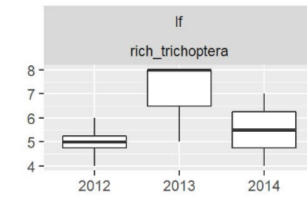
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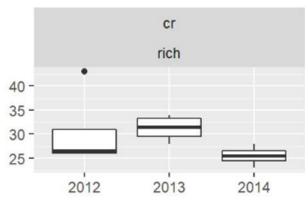
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2013
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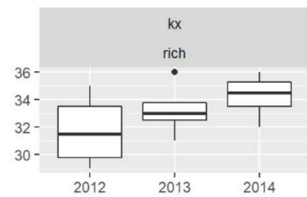
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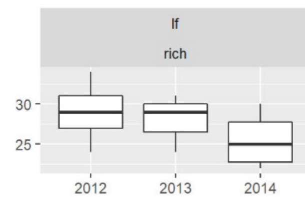
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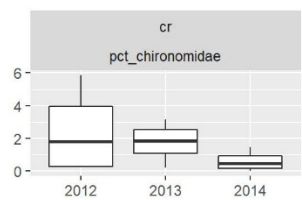
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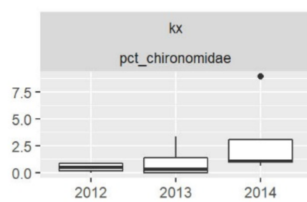
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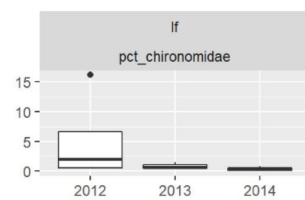
2013
2014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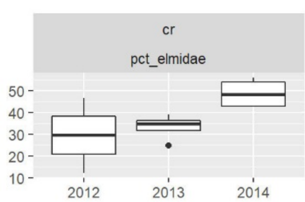
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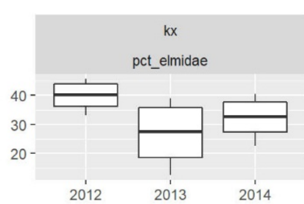
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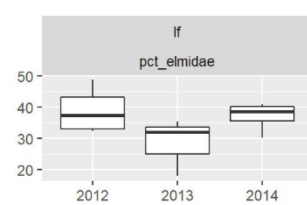
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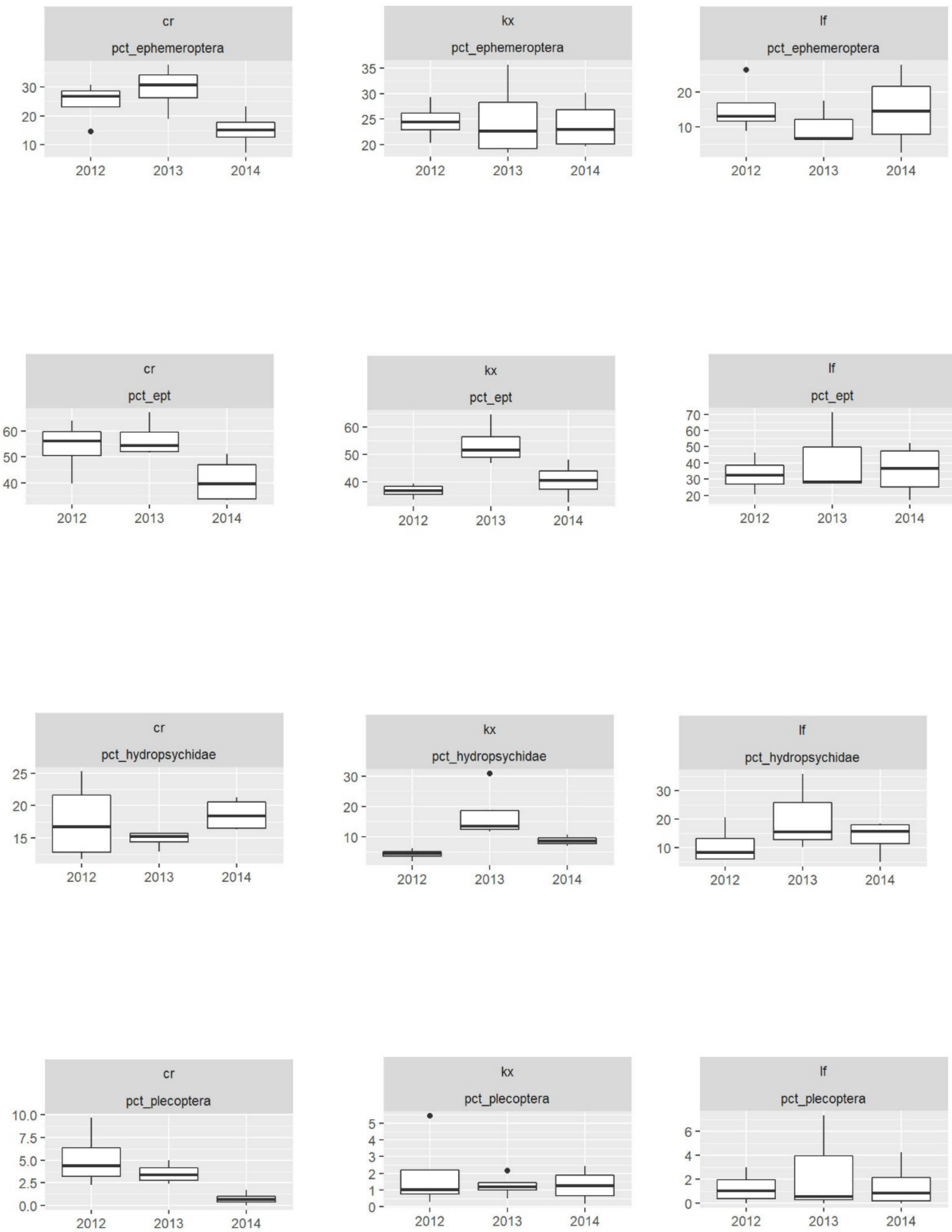
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pct_hydropsychidae
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kx
pct_plecoptera
lf
pct_plecoptera
2012
2013
2014